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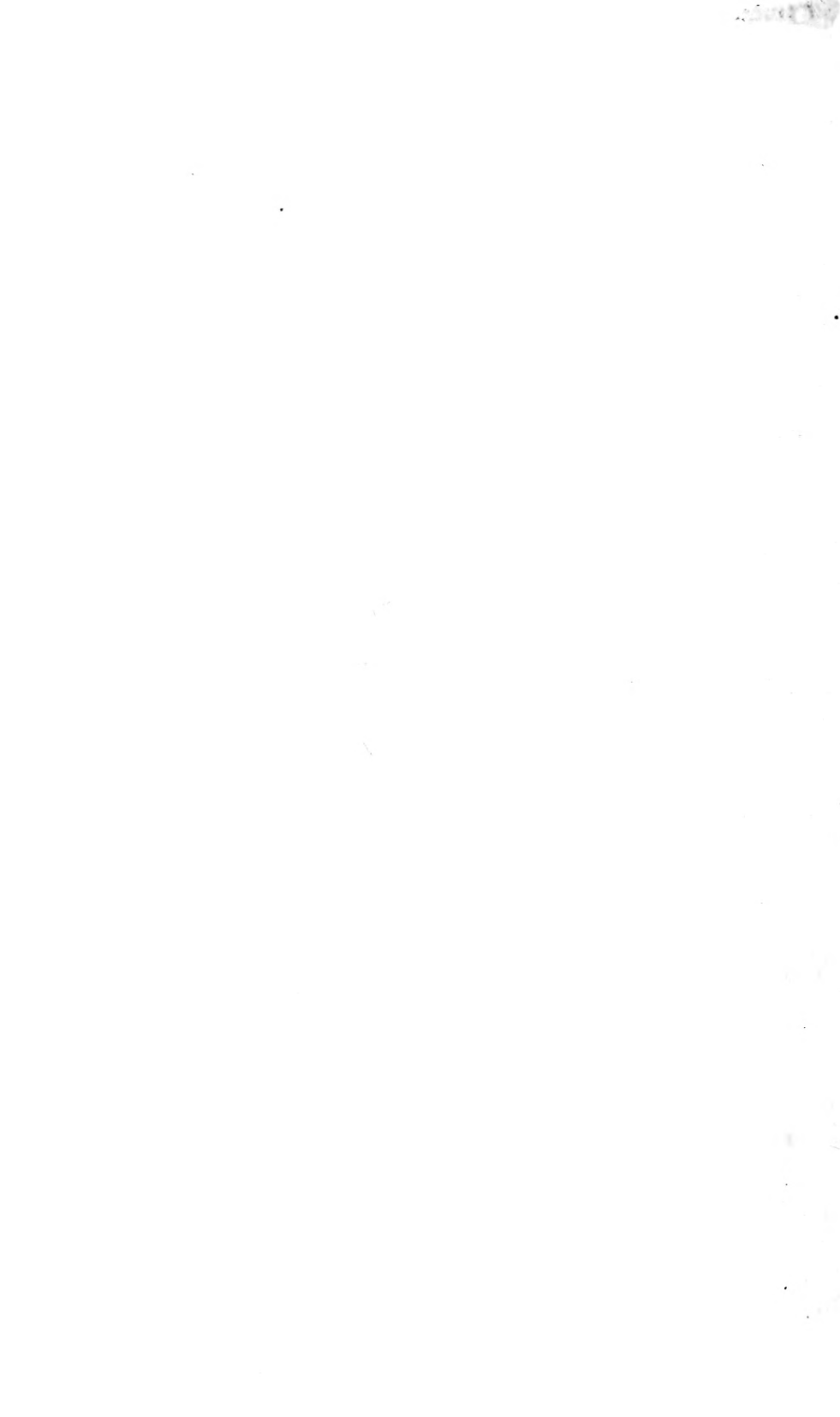
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# STUDIES ON THE HYDROGEN ION CONCENTRATION OF THE SOIL AND ITS SIGNIFICANCE TO THE VEGETATION, ESPECIALLY TO THE NATURAL DISTRIBUTION OF PLANTS.

BY  
CARSTEN OLSEN.

## Introduction.

Since S. P. L. Sorensen in 1909 showed how important the hydrogen ion concentration<sup>1)</sup> is as a factor at the course of enzymatic processes and drew the attention of biologists to the significance which this concentration might be supposed to have for biology on the whole, numerous researches have been made on this question. I. a. can be mentioned the fine researches made by Hasselbalch (1912), Lundsgaard (1912), as also by Hasselbalch and Gammeltoft (1913) on the importance of preserving a fixed and closely limited hydrogen ion concentration in the blood of man and the higher species of animals, and the pointing out of the regulating activities by which the organism will attain this end. On the whole this question has been more thoroughly gone into by the animal physiologists, while the phyto-

<sup>1)</sup> By acidity (or alkalinity respectively) was in older days often meant the total quantity of acid (or base respectively) determined by titration, but the values come to at such an analysis does not need to be in any fixed relation to the hydrogen ion concentration  $\alpha$ ; the real acidity. As will be known, this is only determined by the concentration of the hydrogen ions and hydroxyl ions present and not by the non dissociated parts of the acids and the bases.

The hydrogen ion concentration of a liquid is determined by the number of hydrogen ions expressed in g found in one litre of the liquid, and the number is always expressed as a negative power of 10. Liquids which as pure distilled water contain as many hydrogen ions as hydroxyl ions are said to give a neutral reaction. As the product of the hydrogen ion and hydroxyl ion concentration in an aqueous solution at 18° is  $10 \div 14.24$  (the dissociat-

physiological importance of the hydrogen ion concentration has only been examined to a small degree. In the autumn 1916 when these researches were commenced, only two larger-sized works had been published on the phytophysiological domain, i. e. a dissertation by Jenny Hempel (1916) on the hydrogen ion concentration of the cellular juice of the plants, especially the succulent plants, as also the buffer action on the metabolism of the latter, and a paper by Wagner (1916) on the hydrogen ion concentration in the cellular juice of the higher plants under infection with phytopathogenous bacteria.

The object of the present researches which, as mentioned above, were commenced during the autumn 1916, has been to examine the relation of the higher plants to the hydrogen ion concentration in their natural substratum, the soil. Especially it has been the object to examine whether the hydrogen ion concentration in the soil plays a dominant part as a plant-distributing factor in Nature, and the researches, therefore, are essentially of an ecological nature.

It is likely to think that the hydrogen ion concentration may play an essential part as a plant-distributing factor. Both phyto-geographists and agricultural scientists have long ago noticed that it is of great importance to the plants whether or not the soil contains a greater quantity of calcium carbonate, and that the calcium carbonate effects the different plants differently. On the other hand it has been a matter of dispute on what this effect depends, and not until of late the opinion seems to have gained ground, that it depends essentially on the action effected by the calcium carbonate on the reaction of the soil, i. e. its hydrogen ion concentration, the calcium carbonate giving an al-

ing constant of the water both the hydrogen ion as the hydroxyl ion concentration in pure water at this temperature is  $10 \div 7.07$ . It follows that if the hydrogen ion concentration of a liquid is known, the hydroxyl ion concentration will also be known; consequently it suffices to state the hydrogen ion concentration. Instead of stating the hydrogen ion concentration the logarithm with unlike sign is as a rule stated; this value, called the hydrogen ion index, is indicated as  $p_{H^+}$ , and will thus for pure distilled water be 7.07. If  $p_{H^+}$  is greater than this value, there are more hydroxyl ions than hydrogen ions in the liquid, which is said to react like a base. If  $p_{H^+}$  is smaller than 7.07 there are more hydrogen ions than hydroxyl ions, and the liquid is said to give an acid reaction. Reference is besides made to S. P. L. Sørensen 1909, Höber (1914), Michaelis (1914), and Clark (1920).

kaline reaction to the soil liquid, whereas soil not containing this substance as a rule reacts more or less like an acid.

Elder authors on the other hand entertain essentially different views as to the significance of the calcium carbonate, and on this subject there is a very elaborate literature, which will be briefly mentioned in the following.

It has long ago been noticed that certain plants, the so-called calciphilous plants, only are found on calcareous soil, i. e. soil an essential part of which is calcium carbonate, whereas other plants, the so-called calcifuge plants, or as indicated by many authors silicicolous plants never are found on calcareous soil, but only on soil not containing calcium carbonate, and the main constituent of which therefore is sand (quartz), clay, or humus. As early as 1814 Wahlenberg (1814 p. LX) has drawn the attention to the fact that certain plants exclusively are found on calcareous soil, but Unger (1836) was the first whose attention especially was drawn to the importance of the calcium carbonate to the distribution of plants; thus he gives lists of species only found on calcareous soil and of species only found on soil mainly consisting of schist, granite, or gneiss. Similar studies have been made by Schnitzlein and Frickhinger (1848), Sendtner (1854), Kerner (1863, 1869 and 1898), Nägeli (1865), Contejean (1881), and Adamović (1909). These authors Nägeli excepted, describe the calcifuge plants as silicicolous plants, as these species most often are found on sandy soil. Schnitzlein and Frickhinger as also Sendtner are of opinion that the silicicolous plants need more silicon than the calciphilous plants, the latter more calcium than the silicicolous plants. This opinion is not shared by Kerner, who does not consider the silicicolous plants as plants needing more silicon than other plants, but on the other hand as plants that cannot develop normally when the soil contains calcium carbonate. He founds his opinion on researches showing that high-moor plants which he includes under the silicicolous plants are killed, when constantly watered with water containing calcium carbonate.

However, it gradually appeared that many plants are not as absolutely bound to one or the other form of soil as originally supposed. Thus a species which in one region only is found on calcareous soil, may in another region also be found on sandy soil (Drude 1887). That the struggle for existence with other

species also plays a part appears from the researches made by Nageli (1865) on the appearance of *Achillea atrata* and *A. moschata* both found in the Alps, but not always in the same area. If both species are found in a valley with both calcareous soil and slaty soil, *A. atrata* occupies the calcareous soil, *A. moschata* the slaty soil. If on the other hand only one species is found, this species will occupy both the calcareous soil and the slaty soil. Thus the species are only bound to a certain form of soil when both of them are found and thus may compete with each other for the place. When on the other hand the competition is excluded owing to only one species being found, both species can grow on both forms of soil; however, it is said that *A. moschata* will thrive worse and will be found rarer on calcareous soil than *A. atrata* on slaty soil.

In recent times American investigators (Pipal 1916, White 1916) have made experiments with *Rumex acetosella*, which also have shown the importance of the competition to the distribution of plants. *Rumex acetosella* which is supposed to be lime-avoiding appeared to thrive best on soil containing ample quantities of calcium carbonate. The reason why the plant nevertheless is more frequently found on sandy soil is that it is coming on comparatively better here than most other plants; consequently the struggle for the place with other species will become much less sharp than on limy soil, where the plant actually would thrive best, but it will never or seldom be found here because it is supplanted by other and stronger species.

Attempts to cultivate species which in Nature either exclusively or most frequently are found on calcareous soil have in most cases shown that these species are not thriving well on soil not containing calcium carbonate, and further it has been shown that plants that in Nature never or seldom are found on calcareous soil generally are not coming on well, when cultivated on calcareous soil (Sendtner 1854, Kerner 1863, 1869, 1898, Christ 1879, Roux 1900). When lime-avoiding plants are cultivated on calcium carbonatic soil they will as a rule become chlorotic, leaves and stalks assuming a yellowish or yellowish green colour due to the defective development of the chlorophyll (Gris 1845, Fliche and Grandeau 1873, 1874, Luedecke 1892, 1893, Roux 1900, Dauthenay 1901, Guillon 1895, Guillon and Brunaud 1903, Hilgard 1906, Molz 1908,

Rivière and Bailhache 1910, Pfeiffer and Blanck 1911, Gile 1911, Gile and Ageton 1914, Gile and Carrero 1917, Creydt 1915). Fliche and Grandeau (1873, 1874) thought that the reason of the so-called lime chlorosis was the want of potassium, as they found that chlorotic individuals of the calcifuge trees *Pinus pinaster* and *Castanea vesca* which they had cultivated on calcareous soil, contained much less potassium than normal not chlorotic individuals. Büsgen (1914), however, has substantiated that calcifuge plants as a rule do not suffer from want of potassium when cultivated on calcareous soil. Büsgen found that chlorotic individuals of *Sarothamnus scoparius*, *Calluna vulgaris* and *Digitalis purpurea* cultivated on calcareous soil did not contain less potassium than normal individuals cultivated on sandy soil. The same observations have been made by Gile (Gile 1911, Gile and Ageton 1914, Gile and Carrero 1917) with regard to *Oryza sativa*, *Saccharum officinarum* and *Ananas sativus*.

The reason of the lime chlorosis seems on the other hand, according to researches made by most investigators, to be want of iron, as it appears that chlorotic individuals of lime-avoiding plants, which have been cultivated on calcareous soil contain less iron than normal, not chlorotic individuals of the same species (Schimper 1898, Gile 1911, Gile and Carrero 1917, Gile and Ageton 1914), and that chlorotic individuals may be cured by repeated dosings of the plants with solutions of ferric salts (Gris 1845, Sachs 1888, Guillon 1895, Hiltner 1909, 1911, 1915, Gile 1911, Gile and Carrero 1917, 1920).

Thus it seems to be evident from the researches made by the said investigators, that the lime-avoiding plants have difficulties in absorbing iron on calcareous soil, where iron is found in almost undissolved condition. However, it does not appear from these researches why the calciphilous plants do not suffer from want of iron on calcareous soil, and the researches have shown that calciphilous plants as a rule do not contain less iron than the lime-avoiding plants. The latter thus cannot be considered as plants needing more iron than the lime plants. The studies made on the nature of the chlorosis, therefore, do not explain why the different plants react differently towards calcium carbonate.

Different opinions have been put forward with regard to this

question. That the view taken by the elder phytogeographers that the calciphilous plants need more calcium than the calcifuge species and the latter more silicon than the former is incorrect, has been proved by Hoffmann (1865) and Fliche and Grandeau (1879) who have shown that calciphilous plants as a rule do not contain more calcium than the lime-avoiding ones, nor that the latter in general contain more silicon than the calciphilous plants. It also appeared that calciphilous plants do not thrive on a non-calcium carbonatic soil, even if calcium sulphate is added to the soil (Hartwell and Damon 1914). Thus calcium sulphate has not the same effect as calcium carbonate, and this is indicative that it is not the content of calcium that matters but, on the other hand, the content of carbonate.

A number of investigators have been of opinion that it is not the chemical qualities of the calcium carbonate, but its physical ones that are of vital importance to the plants, e. g. Thurmann (1849) who states that the calcium carbonate gives the soil certain physical qualities, i. a. calcareous soil will easily become dry. Therefore he describes the calciphilous plants as xerophytes and the calcifuge plants (siliculous plants) as hygrophytes, as he thinks that sandy soil in most cases is moist soil. Of latter-day authors Hoffmann (1871), Drude (1887) and Kraus (1911) virtually adhere to this theory.

Of late Truog (Truog 1918, Truog and Meacham 1919, Parker and Truog 1920) have advanced the theory that the calciphilous plants need more calcium carbonate than the calcifuge plants. He supposes that the calciphilous plants have a more active metabolism than the calcifuge ones, and that considerable quantities of acid is formed in the cellular juice at the metabolic process. To neutralize these acids the plants use calcium carbonate, and as the calciphilous plants have a more active metabolism than the calcifuge species, the latter need more calcium carbonate than the former.

Finally, as mentioned above, the stress has — especially in the science of agriculture — been laid on the alkaline reaction given to the soil by the calcium carbonate. One of the first authors who considers the "lime question" as a question of the reaction of the soil, is Ravn (Christensen, Harder and Ravn 1909). He describes the calciphilous plants as plants that are coming on best when the soil gives an alkaline reaction, the calcifuge

plants as species which are thriving best on acid soil; he therefore proposes the designation "calciphilous plants" replaced by "base loving plants" or "basic soil plants" and the designation "calcifuge plants" by "base shý plants" or "acid soil plants". Ravn and later Ferdinandsen (1918) have divided the field weeds — according to their being found most frequently on acid or alkaline-reacting soil, — in acid soil plants and basic soil plants; for the researches of the reaction of the soil they have used both litmus solution and the azotobacter test<sup>1)</sup> introduced by Christensen (1906), Christensen and Larsen (1910).

In phytogeography, studies on the calcifuge moss species *Sphagnum* have lead to the result that the calcifuge nature of

<sup>1)</sup> The azotobacter test does not in reality give any expression of the reaction of the soil, far less of its hydrogen ion concentration. This test is made in the following way.

A certain quantity of the soil which is to be examined is placed in a flask containing a solution of mannite and secondary calcium phosphate; then it is inoculated with a raw culture of the bacterium *azobacter*. If, after having stood for some time the *azobacter* forms a cohesive membrane on the surface of the liquid, the soil is described as "not needy of lime". If the membrane is not formed the soil is on the other hand described as "needy of lime".

When the sample has been placed in the flask, an active formation of acid takes place in the solution (Bondorff 1918). Whether the *azobacter* membrane is then formed depends on the concentration of hydrogen ions of the liquid not exceeding a value corresponding to 6.7 in  $p_{H^+}$ , as *azotobacter* only develops, if the hydrogen ion concentration does not reach the critical value (Bondorff 1918). This again depends partly on the basic quantity of the sample partly on the acid quantity formed by the bacteria. The latter quantity is hardly the same in all cases as the acid quantity formed surely is effected by accidental circumstances. The objection may of course be raised that the acid quantity formed shall not be the same in all cases, as it has to correspond to the production of acid which under natural conditions takes place in the soil, and which certainly differs as regards the different soils. The *azotobacter* test has then to prove whether a sufficient basic quantity corresponding to an acid production in the soil is found or not. It must be remembered that the conditions of the nutrient liquids are quite different from those in the natural soil and very favourable to the acid producing bacteria, and it is very improbable that what takes place in the nutrient liquids is in any relation to what takes place in the soil under natural conditions. Thus the nitrificating bacteria often act quite otherwise in nutrient liquids than in the soil (vide e. g. Hesselmann 1917, p. 320).

It will appear from what has been developed that the *azobacter* test cannot throw light on the concentration of hydrogen ions in the soil nor, as is maintained by Bondorff (1918), elucidate anything on its buffer content (vide footnote p. 26).

these mosses is due to the fact that they cannot develop in other liquids than such as give an acid reaction, while they are killed by such as have an alkaline reaction, e. g. by calcium carbonatic water (Paul 1906, 1908, Skene 1915). Solutions of calcium sulphate are on the other hand harmless to these mosses (Paul 1906). That calcium carbonate as also basic substances on the whole have a killing effect on *Sphagnum* plants is due to the fact that the acid<sup>1</sup>) slightly soluble found in the cellular walls of these mosses is neutralized, which involves the death of the plants (Leiningen 1907, Paul 1908).

That the reaction of the culture medium also plays a part as regards other mosses appears from the researches made by Kessler (1914) on the germination of moss spores in liquids, which give an acid, neutral, or alkaline reaction. These researches showed that spores of mosses from acid soil, e. g. from high moors or from raw humus only were germinating in an acid liquid, the spores of the calciphilous mosses only in an alkaline one. The researches only concerned the germination itself, which was controlled microscopically.

As regards the higher plants there have — apart from the above mentioned studies on weeds — only been made few researches on their relation to the reaction of the soil. Chodat (1915) drew the conclusion, based upon researches made with the calcifuge species *Digitalis purpurea*, that the defective ability of this plant to develop on calcareous soil is due to the fact that it cannot thrive in alkaline soil as not only calcium carbonate, but also other substances which have an alkaline reaction check the growth of this plant when mixed with the soil.

While studying the appearance of *Molinia coerulea* and *Eriophorum vaginatum* in the English moors, Jefferies (1915) found that *Eriophorum* always is found on more acid soil than *Molinia* (the "acidity" of the soil was determined by titration).

<sup>1</sup> Baumann and Gully (1910) hold that *sphagnum* plants do not contain any acid, but that the acid reaction is produced by the colloid cellular membranes adsorbing the bases of neutral salts and thus liberating the acids. It is these acids which give the acid reaction, while the membranes themselves originally do not contain any acid. Their theory also includes the humus substances, which they do not either consider as acids. Baumann and Gully's colloid chemical theory has, however, proved to be incorrect, as Odén (1919 p. 140) has shown that neither *sphagnum* plants nor humus are able to decompose neutral salts.



Furthermore after the beginning of the researches published in this treatise, Wherry, an American author (1918) published an essay on an examination of the habitats of 40 orchids with regard to the concentration of hydrogen ions in the soil. He came to the conclusion that some species (the calciphilous plants) only grow on soil giving a neutral or an alkaline reaction ( $p_{H-}$  7—8), others only on strongly acid soil ( $p_{H-}$  4—5), others again only on less acid soil, while some few species were not bound to soils with a narrowly limited hydrogen ion concentration<sup>1)</sup>. Wherry has later (1920 II) in the same way and with similar results examined the habitats of the halophytes. An earlier treatise by Wherry (1916) deals with the habitats of the fern species *Camptosorus rhizophyllus*. This species which is supposed to be calciphilous appeared however not at all to be exclusively bound to a soil giving a neutral or an alkaline reaction.

It may finally be mentioned that also Arrhenius (1920) has measured the hydrogen ions in soil taken from the habitats of some Swedish formations. He found that the concentration of hydrogen ions in soil within the same formation lies within fixed ranges of hydrogen ion concentration more or less narrowly limited.

From the above literature it will appear that there is every probability that the concentration of hydrogen ion in the soil plays an essential part as a plant distributing factor in Nature, and the object of the researches published in the present treatise has, as mentioned before, been to examine what significance might be attached to the concentration of hydrogen ions in the soil in the said respect.

In order to examine this question, I have by aid of Raunkjær's formation-statistical method made a number of researches of the composition of natural Danish plant formations<sup>2)</sup>. Simultaneously I have drawn samples of soil on the habitats, the hydrogen ion concentration of which was determined. By comparing the analysis of the botanical formations with the physico-chemi-

<sup>1)</sup> The value of Wherry's investigations is partly deteriorated owing to the procedure used at the determination of the hydrogen ion concentration (vide p. 23).

<sup>2)</sup> The word "formation" is in this treatise used to designate the smallest phytogeographical unity; all plant communities the specific composition and appearance of which are essentially alike and the dominating species of which are the same belong to the same formation.

cal soil analysis there appeared — as will be shown later — on an immediate view to be a rather fixed law-directed relation between the composition of the vegetation and the concentration of hydrogen ions in the soil, as essential variations in the latter always are accompanied by variations in the composition of the vegetation, also provided the other conditions were alike, while habitats with nearly the same concentration of hydrogen ions and the same conditions of light and moisture have approximately the same vegetation. By statistical treatment of the material it furthermore appeared that the single species only are found on soil the hydrogen ion concentration of which lies within a fixed range of hydrogen ion concentration, different for each single species.

However, it was not my sole object to substantiate this, but also to examine whether it is the hydrogen ion concentration as such which determines the plant distribution as it has been shown. For it may be a question partly of direct, partly of indirect actions of the hydrogen ion concentration. In case the concentration of hydrogen ions in the soil in a number of habitats varies regularly with the composition of the vegetation, the different plants will thrive best at hydrogen ion concentrations different for the different species, if it is the hydrogen ion concentration which is the direct reason of the plant distribution. To examine whether this was the case, I have made experiments with water cultures of different species, these species being cultivated in solutions of different hydrogen ion concentration under conditions which were otherwise homogeneous.

As it is, it may also be a question of indirect actions of the concentration of hydrogen ions. Thus it is a well-known matter that the concentration of hydrogen ions in the soil (reaction) exercises a rather great influence on its microorganic life and the transformation processes (Bear 1917, Noyes and Conner 1919, H. Fischer 1909, Potter and Snyder 1917). It is especially the influence which the reaction of the soil exercises on the course of the transformation processes of the nitrogen which may be supposed to play a part at the plant distribution, for of all the substances the transformation of which depends on the activity of the microorganisms only nitrogen is of importance to the higher plants. It has in many cases been substantiated that nitrification does not take place in strongly acid soils, and

that the nitrification is highly furthered when calcium carbonate or other substances, reacting like an alkali are mixed with a soil originally not giving a neutral or alkaline reaction (Lemmermann, Fischer and Husek 1909, Behrend 1881, Noyes and Conner 1919, Bear 1917). Bear is of opinion that when calciphilous plants do not thrive on strongly acid soils, it is due to these plants not standing very acid reaction, but on the other hand they demand the nitrogen in the form of nitrate and cannot make use of ammonia nitrogen, which as contrasted to nitrate nitrogen is found in ample quantities in strongly acid soils. On the other hand plants thriving on strongly acid soils can make use of ammonia nitrogen. According to Bear, lime-loving plants = nitrate plants, lime-avoiding plants = ammonia plants. Similar opinions are met with in the forestal literature. Thus Müller (1918) holds that the special qualities of the raw humus and heath soil, which are unfavourable to the wood vegetation are not due to the strongly acid reaction of these soils, but to the absent or highly restrictive nitrification. Hesselmann, the Swedish investigator (1917) who attaches great weight to the significance of nitrification for the plant distribution, entertains nearly the same view. He too considers the plants found on strongly acid, therefore not nitrifying soils as plants which can make use of ammonia nitrogen, while the plants found on less acid, neutral, or alkaline soils are such as demand nitrate as the source of nitrogen.

In order to examine whether the more or less complete transformation of the nitrogen is of importance to the plant distribution, I have taken samples on some habitats of the formations investigated to examine the course of the forming of ammonia and that of the nitrification. I have also made experiments on the possible different value of ammonia nitrogen and nitrate nitrogen as source of nitrogen for the different plants; for these experiments I have partly used plants exclusively found on strongly acid not nitrifying soil, partly plants exclusively found on soil giving poor acid, neutral or basic reaction, the nitrification as a rule being most active in such soils.

Another indirect action of the concentration of hydrogen ions in the soil upon which the stress also has been laid, is the influence exercised by the hydrogen ion concentration on the solubility of aluminium, as small quantities of aluminium in solution

may be found in acid soils (Abbott, Conner and Smalley 1913, Ruprecht and Morse 1917, Kappen 1916, 1917). Hartwell and Pember (1918, 1919) hold that this is the reason of lime-loving plants not thriving on strongly acid soils, as aluminium compounds even in small quantities are said to be toxic to them, whereas lime-avoiding plants are not or only to a small degree injured by aluminium compounds in solution. The theory has been advanced on the basis of water culture experiments with rye (*Secale cereale*) and barley (*Hordeum distichum*), which showed that aluminium compounds are toxic to the lime-loving plant barley, while rye which thrives comparatively well on strongly acid soil, is almost entirely unsusceptible hereof. According to the two authors the concentration of hydrogen ions in the soil does not play any direct part, as they are of opinion that barley can stand as high concentration of hydrogen ions as rye.

In order to test this theory and thus examine whether aluminium is more toxic to lime-loving plants than to lime-avoiding plants, I have made some water culture experiments partly with lime-loving plants, partly with lime-avoiding plants.

A third indirect action of the concentration of hydrogen ions in the soil, which may be supposed to bear a part as regards plant distribution, is the influence exercised by the concentration of hydrogen ions on the contents of natural plant nutrients of the soil, as some nutrients in acid soil easily will dissolve and gradually be washed out. Therefore, acid soils are often poorer on mineral plant nutrients than neutral and alkaline ones (Hoagland 1917, p. 557).

I have in order also to have my attention directed to the possible significance which this may have on plant distribution determined, as regards some of the examined habitats, the contents of easily soluble mineral plant nutrients of the soil.

Furthermore it may be possible that the plants in Nature gradually not only form the hydrogen ion concentration most favourable to them, but on the whole form the soil condition most favourable to themselves. The composition of the vegetation would in this case become the primary, the concentration of hydrogen ions the secondary. However, I want to observe that this is highly improbable, as all agricultural experience decidedly tells against it. In case this theory was correct it would

be most paying year after year to cultivate the same species on the same soil, but as is well-known, this very procedure has proved unprofitable, consequently everywhere rotation is now used. It is very probable that the single species change the conditions of the soil in a direction unfavourable to themselves. In order to try whether the plants themselves are able to form a concentration of hydrogen ions favourable to themselves, I have made experiments with a view to examining whether the plants are able to modify the concentration of hydrogen ions of a culture medium, and this being the case whether the modifications take place in a direction favourable to the plants and whether the different species change the concentration of hydrogen ions to different values.

As will appear from the above, my investigations comprehend both analysis of formations and their habitats as culture experiments. It will, therefore, answer the purpose in the first section (A) to mention the habitats and their vegetation and in the second section (B) to mention the experiments.

## A. THE HYDROGEN ION CONCENTRATION OF THE SOIL AND THE DISTRIBUTION OF THE SPECIES (FORMATIONS).

### **Methodics of the Research.**

**Selection of objects experimented on.** The researches comprise meadow and wood ground formations as also some formations on open mineral soil.

For the investigations I have as far as possible selected such formations as might be supposed to be in biological equilibrium with the conditions given at the moment of formations the composition of which is comparatively constant as long as the conditions are not changed. To bring about this it is not at all necessary, that the formations shall not be affected by cultural intrenchment. Thus the meadow formations are highly affected by human activity, as they are exposed to cutting, but this has been the case through many years, and consequently the vegetation has had time to adapt itself to the conditions created by

the cutting. While the meadow formations may be said to be in comparative equilibrium with the conditions given at the moment, this only to a smaller degree applies to the wood-ground formations. The reason hereof is mainly that the conditions of light in the wood-ground owing to fellings constantly being made very seldom is the same for a number of years. Therefore the wood-ground vegetation does not get time to adapt itself to the given conditions of light, before they change again. For this reason the wood-ground formations examined have as far as possible been selected in places, where it might be supposed that fellings have not been made of late years.

Furthermore stress has been laid upon the single formations examined being severally homogeneous, and therefore it was necessary only to examine small areas, in most cases areas of only a few square metres. This also was made in view of selecting soil samples for examination, as it was not considered correct to allow the analysis to "pass" for too great areas.

**The formation-statistical method.** When making researches as the present ones, it is of great importance to determine the frequency of the species, as the degree of frequency must be considered as implying the ability of the plants to assert themselves under the given conditions, so that a greater frequency in one species indicates a greater ability in the species to stand the ground at the given habitats than a less frequency. Therefore, as has been mentioned before, Raunkjær's formation-statistical method<sup>1)</sup> has been used at the botanical analysis of the formations. Only the frequency of the species (percentage) has been determined and owing to the small areas examined, only 10 trial areas have been selected in each locality.

**The determination of the hydrogen ion concentration of the soil samples.** In a depth of about 10 cm. samples of the soil were taken on each of the habitats examined, as a rule two or three in each locality. These two or three samples were mixed at the spot and cleared of pebbles, sticks and the like. These samples were carried home in a glass holding about

<sup>1)</sup> When using this method a number of trial areas, each of 0.1 m<sup>2</sup> are selected of the formation which is to be examined. By stating for each species the percentage in which it is found in the selected trial areas, figures are come at which express the frequency of the species in the formation. See further Raunkjær 1909, 1916, 1918.

70 cc of soil. The samples were transferred to beakers and 70 cc of distilled water added; they were allowed to stand for about 24 hours, repeated stirrings being made during this time with a glass spatula. After the lapse of the 24 hours the hydrogen ion concentration was measured. For the determination hereof I have partly used the electrometric method, partly the colorimetric one.

*The electrometric method* was executed according to S. P. L. Sørensen (1909). For an electrode vessel I have used the apparatus designed by Hasselbalch (1911), as the carbonic acid when using this apparatus is not driven off from the liquids, as was the case at the original measurements. The soil extracts which were measured electrometrically were produced by pouring the soil depositions through a china strainer the holes of which were hardly one mm in diametre. Thus the coarsest particles were removed, as otherwise particles might choke the glass tubes of the electrode vessel and the perforations of the stop-cock. The soil extract thus strained was introduced into the electrode vessel and the measurement took place in the ordinary way.

The measurements do not occasion special difficulties apart from the fact that owing to the small electrical conductive power of the liquid only small turns of the quicksilver column of the capillary electrometre are obtained, and therefore it is not possible to measure with as great exactitude as is otherwise the case. For this reason I have only measured to one decimal place when giving  $p_H$  values, as only this place might be accurately determined. Besides a determination of  $p_H$  with an exactitude of  $\pm 0.1$  is quite sufficient when measuring the concentration of hydrogen ions of the soil extract.

*The colorimetric method*, elaborated and described by S. P. L. Sørensen (1909) distinguishes itself in contradiction to the electrometric one by its great simplicity, as very few apparatuses are required. It is extremely quick and easy to work, and because it is especially adapted to measurements of soil extracts, it will briefly be described here.

At this method indicators are used for the determination of the concentration of hydrogen ions. An indicator is a substance which in liquids of different hydrogen ion concentration — which, however, must lie within a certain range of hydrogen ion concentration, fixed for each indicator ("the range" of the indicator)

— assumes different colours or tones. The concentration of hydrogen ions of a liquid may be determined by aid of an indicator in the following way, the concentration of hydrogen ions lying within the range of the indicator.

A number of standard solutions (liquid of comparison), the hydrogen ion concentration of which is known and varies with fixed intervals (e. g. with a reciprocal difference of 0.2 in  $pH$ ) over the range of the indicator are measured in test tubes, equal volumes of liquid being placed in each test tube. The same volume of the liquid the hydrogen ion concentration of which is to be measured is also introduced into the test tube. Equal quantities (a few drops) of the indicator solution are then added to each test tube. The colours of the standard solution will then form a colour scale, in which the liquid the hydrogen ion concentration of which is to be determined may be ranged, and the latter will then have the same hydrogen ion concentration as the standard solution the colour and tone of which are the same. The standard solution and their preparation have been described in the work published by S. P. L. Sørensen in 1909.

When using the colorimetric method for the determination of the hydrogen ion concentration of the soil extract it is of course necessary to filter the soil extracts, and it appeared necessary for this purpose to use washed filters, such filters of course not containing any acid at all. The Swedish Berzelius filters proved excellent in this respect, and consequently I have always used them. Furthermore it appeared to be necessary to filter the soil extracts quite clear. This was most easily obtained by transferring as much as possible of the solid soil to the filter. If the soil extract is not filtered absolutely clear, the measurement will give wrong results, which is due to the fact that the indicator is absorbed by the particles floating in the liquid. In order to avoid that a possible absorption might affect the hydrogen ion concentration of the extract, the filtrate first run through was not used at the measurements.

The clear soil extracts are often brownish or yellowish from the organic substances in solution which render the comparison of the tone of the indicator in the soil extract with its colour-tone in the different standard solutions difficult, in many cases even impossible. This difficulty, however, was easily surmounted



when making the comparison in a comparator, the construction of which will be seen from the following figure.

In the first row the test tubes with the standard solutions are placed to which the indicator solution is added; in this row is also placed a test tube containing the soil extract, the hydrogen ion concentration of which is to be determined to which is also added the indicator solution. In the second row a test tube containing the soil extract but without the indicator solution is placed behind each of the standard solutions. Behind the test tube in the first row which contains soil extract + indicator a test tube is placed containing distilled water. If the standard solutions are now looked at through the "window" cut in the foremost vertical wall of the support the different colours of the indicator in the different standard solutions will be seen against the colour of the soil extracts, the confusing effect of which during the measurements thus is eliminated; consequently it will be easy to range the

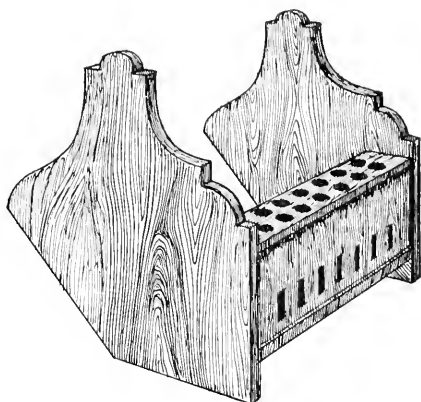


Fig. 1. Comparator.

test tubes containing soil extract + indicator in its proper place in the colour scale. When using this procedure it is possible to determine a concentration of hydrogen ions of the soil extract of any colour whatever. As the soil extracts always are at least slightly brown coloured the comparator has been used at all the measurements; besides, it ought always to be used when making colorimetric measurements of the concentration of hydrogen ions of the soil extract. It is a matter of course that the test tubes must be colourless, of the same thickness and all provided with equal volumes of liquid. The test tubes used by me were 14 mm in external diameter and about 13 cm high, 4 cc of liquid were used in each glass.

It was combined with the greatest difficulty to find suitable indicators, as it quickly appeared that far the greater part of all known indicators were inapplicable, as wrong results, i. e. results

not in conformity with the electrometric measurements, are reached. Thus all the indicators mentioned by S. P. L. Sorensen in 1909 which owing to the position of the change range might be considered, proved to be inapplicable as errors of up to 2.0 in  $p_H$  were reached when using them. The reason hereof is supposed to be that these indicators combine with the substances found in the soil extract or are decomposed by them. In many cases the indicator is completely decolorized by the soil extract. This e. g. holds good of litmus which in many soil extracts assumes a yellow or yellowish red colour<sup>1</sup>). Only one of the indicators at hand at the Carlsberg Laboratory in 1916 proved suitable, i. e. naphthol-phthalein, described by S. P. L. Sorensen and Palitzsch (1910). American investigators (Gillespie 1916, Gillespie and Wise 1918) have also substantiated that most of the existing indicators are inapplicable for the determination of the concentration of hydrogen ions in the soil extracts. Gillespie (1916), however, found that a number of indicators prepared by Lubs and Clark (1915) were suitable. Not until I have had these indicators ordered from America I succeeded in obtaining satisfactory results by aid of the colorimetric method.

I have made use of 4 indicators at the colorimetric measurements; their names, colour change and range will appear from the following tabular survey (table 1).

The three first mentioned indicators originally prepared and described by Lubs and Clark (1915; see also Clark and Lubs 1917) were used in alcoholic solutions, prepared by dissolving 0.4 g of the indicator in one litre of 93 per cent alcohol; 0.4 g of naphthol-phthalein was dissolved in 600 cc of 93 per cent alcohol, then 400 cc of water were added. Two drops of the solutions of bromfenolblue and bromcresolpurple were used for each test tube at the measurements, three drops of the bromtymolblue solution and finally 5 drops of the naphthol-phthalein solution. This

<sup>1</sup> Christensen and Larsen (1910) describe soil samples as very acid when giving a litmus solution a yellowish red colour. As is well known a litmus solution will never turn yellowish red when adding hydrochloric acid but only red. A clay soil sample examined by me assumed when adding litmus solution a yellowish red colour; at an electrometric measurement the hydrogen ion concentration of this extract was found to be 6.7 expressed in  $p_H$ . Thus it appears that nearly neutral soils may easily at the litmus test be described as "very acid".

Table 1.

Chemical name	Common name	Colour change	Range $p_{H^+}$
Tetra-bromo-phenol-sulfon-phthalein	Brom-fenol-blue	yellow-blue	3.0—4.8
Dibromo-orto-cresol-sulfon-phthalein	Brom-cresol-purple	yellow-purple	4.8—6.8
Dibromo-thymol-sulfon-phthalein	Brom-tymol-blue	yellow-blue	6.0—7.6
$\alpha$ -Naphthol-phthalein	Naphthol-phthalein	light reddish-green	7.2—8.7

standard solution used at the measurements were those generally used at the Carlsberg Laboratory (vide S. P. L. Sørensen 1909).

At the measurements of the soil extracts I have made use of altogether 13 different standard solutions, each of which had the following  $p_{H^+}$  value: 3.2 : 3.6 : 4.0 : 4.4 : 4.8 : 5.2 : 5.6 : 6.0 : 6.4 : 6.8 : 7.2 : 7.6 and 8.0. By the aid of four indicators four different colour scales were laid down, the bromfenolblue being used in the range 3.2—4.8 bromcresolpurple in the range 4.8—6.4, bromthymolblue in the range 6.0—7.6 and finally naphthol-phthalein in the range 7.2—8.0. As will be seen, the range of the two above mentioned indicators do not interfere with one another. Therefore, it was not quite easy to determine the concentration of hydrogen ions of a soil extract having a  $p_{H^+}$  value in the vicinity of 4.8. After some time, however, I succeeded in determining the hydrogen ion concentration in the extracts with sufficient exactitude in this range, but only if both indicators were tried. In the said difficult range (near  $p_{H^+}$  4.8) American investigators make use of methyl red (vide Gillespie 1916), but when using this indicator I have come to wrong results, as methyl red just as litmus is decolorized in many soil extracts, especially in those taken from very humic soils.

As appears from the above, the series of standard liquids used had a difference of 0.4 in  $p_{H^+}$  as regards the concentration of hydrogen ions. If it was thought that an extract with added indicator had its proper place in the colour scale between two subsequent standard liquide, it was given the  $p_{H^+}$  value 4.2, if the tone was e. g. between that of the two standard liquids the  $p_{H^+}$  of which was 4.0 and 4.4 respectively. If on the other hand its tone was nearer to that of the standard liquid the  $p_{H^+}$  value

of which was 4.0, it was given the  $p_{H^+}$  value 4.1 e. t. c. In this way it was possible with comparative exactitude to discern between differences of 0.1 in  $p_{H^+}$ . Furthermore it must be stated that all colorimetric measurements have been executed by day, and that the standard solutions in the test tubes to which the indicator was added only were used the day on which they were prepared. This was necessary, as three out of the four indicators are not durable in the standard solutions; bromfenolblue is the least durable, as its colour-tone in the standard solutions quickly will increase when standing for some time. It may even be necessary to renew the standard liquids containing this indicator several times in the course of the same day. Bromcresolpurple and bromthymolblue on the other hand are bleached when standing for same time, while bromthymolblue seems to be rather durable. The alcoholic indicator solutions will on the contrary last a long time.

Sometimes it may occur that one  $p_{H^+}$  value is reached for a given soil extract when using bromcresolpurple, another value when bromfenolblue is used. It is e. g. sometimes found that soil extracts measured with bromcresolpurple has a tone lying between that of the two standard liquids, the  $p_{H^+}$  of which is 4.8 and 5.2 respectively, while it has been found when bromfenolblue is used, that the soil extract has a tone lying between that of the two standard liquids, the  $p_{H^+}$  of which is 4.4 and 4.8 respectively. In this case it has always appeared that the  $p_{H^+}$  value indicated by bromcresolpurple is in nearest conformity with the  $p_{H^+}$  value found by electrometric measurements. At colorimetric measurements bromcresolpurple ought always to be the first indicator tried. This is besides also practical from the reason that in case the  $p_{H^+}$  value of the soil extract does not lie within the change point of this indicator, it is known at once whether bromfenolblue or bromthymolblue is to be used. Using naphthol-phthalein which is rarely used, a  $p_{H^+}$  value will sometimes be come at, which is lower than the  $p_{H^+}$  value reached by the aid of bromthymolblue. In this case it has always appeared that the  $p_{H^+}$  value reached by aid of bromthymolblue is in closer conformity with the electrometrically measured value than the  $p_{H^+}$  value reached by aid of naphthol-phthalein.

**Comparison between the results of the electrometric method and those of the colorimetric one.** When the colori-

metric measurements are executed with careful observation of the rules laid down here, sufficiently exact results, i. e. results conformable to the electrometric measurements are reached. The conformity between the results of the methods will appear from table 2, in which the  $p_{H^+}$  values for a number of soil extracts measured by aid of both methods are stated. The measurements comprise both peaty soils and mineral soils. In the table only few of the soil sample analysis are stated, which have been carried out by aid of both methods. Thus the soil samples from all the meadow examined have been measured by both methods. On account of lack of space only the results of 18 double analysis are given, selected at hazard.

It will appear from the table that I have never substantiated greater difference than 0.2 between the results of the two methods as regards the meadow soil and wood soil mentioned in this treatise<sup>1</sup>). Furthermore it will be seen that the colorimetric method, provided it does not give the same  $p_{H^+}$  value as the electrometric one, in most cases will give a somewhat lower  $p_{H^+}$  value.

If the above rules for the application of the colorimetric method on soil extracts are not observed,  $p_{H^+}$  values will be reached which deviate considerably from those found by aid of the electrometric method. If essentially more than two drops of the bromfenolblue and bromcresolpurple solutions are used at the measurements,  $p_{H^+}$  values will be reached for the soil extracts which are too low, because the two said indicators are acids. Of the other two indicators more solution may be used, as this has not proved to affect the result. If the soil extracts are not filtered absolutely clear, too low  $p_{H^+}$  values will be reached<sup>2</sup>).

Gillespie (1916) who has measured the hydrogen ion concentration of soil extracts both electrometrically and colorimetrically, has found similar conformity — though not so good — between the results of the two methods as indicated above. Besides the following investigators have made determinations of

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<sup>1</sup>) As regards arables I have found greater deviations; the difference might here sometime amount to 0.5 in the  $p_{H^+}$  value.

<sup>2</sup>) When executing the litmus test a neutral salt, e. g. magnesium sulphate is often added to the soil precipitate in order that the extract might more easily be made clear (see Christensen and Larsen 1910 p. 431). This, however, might bring about an increase of the hydrogen ion concentration and therefore cannot be permitted.

Table 2.

The numbers of the localities refer to the tables 4—12 and 18—26).

Number of locality	pH· electrometric	pH· colorimetric	Difference
80	3.5	3.5	0
4	3.9	3.8	0.1
104	4.2	4.1	0.1
12	4.4	4.4	0
18	4.7	4.7	0
126	4.7	4.6	0.1
152	5.2	5.0	0.2
26	5.4	5.3	0.1
178	5.6	5.6	0
39	5.7	5.7	0
46	6.2	6.0	0.2
211	6.4	6.4	0
58	6.7	6.7	0
228	6.8	6.7	0.1
67	7.2	7.3	÷ 0.1
241	7.2	7.2	0
76	7.8	7.7	0.1
266	7.9	7.8	0.1

the hydrogen ion concentration of soil extracts: Saidel (1913), G. Fischer (1914), Kappen (1916, 1917, 1920), Kappen and Zapfe (1917), Gillespie (1916, 1920), Gillespie and Wise (1918), Gillespie and Hurst (1917, 1918), Wherry (1916, 1918, 1919, 1920), Sharp and Hoagland (1916, 1919), Hoagland and Sharp (1918), Hoagland and Christie (1918), Plummer (1918), Morse (1918), Olsen (1918), Odén (1919), Lipman (1919), Joffe (1920), Blair and Prince (1920), Martin (1920) and Arrhenius (1920)<sup>1</sup>). The methods of these investigators do

<sup>1</sup>) Wherry's and Arrhenius' investigations have been mentioned in the introduction. The investigations made by the other investigators deal partly with the physical-chemical relation of the humus substances, partly with the influence of the fertilizers on the hydrogen ion concentration of the arable soils, partly with the appearance of potato scab and other similar subjects disconnected with the subject of this treatise. Therefore, they will not be mentioned further here.

not essentially differ from those employed by me. The procedure used by Wherry is the only one that will be more fully entered upon.

As mentioned in the introduction, Wherry has made researches on the concentration of hydrogen ions in the soil on the habitats of American orchids. He used the colorimetric method, and as the determinations were made in the field he had to simplify the method. Besides he has in a special paper in details described the procedure followed by him. He did not extract the soil with distilled water, but with water found in Nature (spring water, river water, or sea water) which, as will be known, in many cases can be very calcareous. After having shaken soil and water in a glass, he allowed this to remain untouched for a few minutes in order that the soil may have time to settle; then the liquid was poured out. He did not filter this liquid which in most cases certainly must have been very turbid, in a few cases even intransparent, but he directly added a similar volume of the indicator solution. He did not use standard solutions, but he only observed the colour of the indicator in the soil extract. Then he roughly stated the concentration of hydrogen ions. Wherry (1920) states that he intends further to simplify the method. It cannot be denied that the procedure followed by him is extremely simple, but the results reached in this way are in return less valuable. By using Wherry's procedure I have substantiated errors of up to 2 in  $p_H$ . The fact that the soil extracts are not filtered is the essential reason of the errors, which might occur when using this procedure. The error is especially great as regards the more acid extracts, while the errors only are small as regards the neutral or basic extracts.

**The significance of carbonic acid on the concentration of hydrogen ions of the soil extracts.** By means of the colorimetric method I have examined how great is the significance of carbonic acid in the soil extracts on the concentration of hydrogen ions. It appeared at these investigations that the concentration of hydrogen ions in the soil extracts decreases, when the carbonic acid is driven off by conducting air not containing carbonic acid through the soil; this, however, only holds good when the  $p_H$  values of the extracts are greater than 4. The  $p_H$  values of soil extracts the hydrogen ion concentration of

which is in proximity of the neutral point is shifted about  $\pm 0.6$  when the carbonic acid is removed. In case the hydrogen ion concentration of the soil extracts is greater, the  $p_{H^+}$  value is altered to a smaller degree when removing the carbonic acid. Soil extracts the  $p_{H^+}$  value of which is near 5 are thus altered about  $\pm 0.2$ . Thus it appears that the soil extracts must not stand for a longer time before the measurements are made, as otherwise carbonic acid will escape.

At a series of experiments with a few neutral or alkaline soils  $p_{H^+}$  was electrometrically determined partly after the soil, to which was added water, had been standing for 24 hours (the general procedure), partly after a current of atmospheric air had been passed through the mixture of soil and water for 24 hours and finally partly after a current of atmospheric air not containing carbonic acid had passed through the mixture of soil and water, also for 24 hours. The following  $p_{H^+}$  values were found as regards a very alkaline soil:

Treated in the ordinary way.....	$p_{H^+} = 8.0$
Atmospheric air being passed through.....	„ = 8.2
Atmospheric air not containing carbonic acid being passed through.....	„ = 8.3

If the soil deposition treated in the ordinary way was filtered, the  $p_{H^+}$  value 8.0 was also reached, as far as the filtered extract was concerned, but when a current of atmospheric air not containing carbonic acid was passed through the filtered extract for 24 hours,  $p_{H^+}$  was found to be = 9.0. It appears from these experiments that almost the same  $p_{H^+}$  values are reached by the procedure used by me, as those reached when the soil depositions are brought in equilibrium with the tension of the carbonic acid of the air, or when the carbonic acid is completely removed. As regards the filtered extract it makes an important difference — especially as regards the neutral and alkaline extracts — whether the carbonic acid is removed or not. It must be presumed that the solid particles of the soil depositions act like a buffer and work the comparatively small significance of the carbonic acid for the  $p_{H^+}$  value.

**Extraction with different volumes of water.** Experiments have been made to show the influence on the concentration of hydrogen ions of the soil extracts in case the relation between



soil and water was varied. The concentration of hydrogen ions of the extract appeared almost to be the same, whether the extract was the result of extracting 70 cc of soil with 70 cc of water or the double volume hereof.

**The hydrogen ion concentration of the soil liquid as compared with that of the soil extract.** As mentioned above I have at my researches determined the hydrogen ion concentration of an aqueous extract. When extracting with water, the soil liquid is of course very much diluted and consequently it cannot be expected that the concentration of hydrogen ions measured in the extract is the same as that found in the soil liquid. Therefore, the hydrogen ion concentration measured in extracts cannot be considered as the real hydrogen ion concentration of the soils (the soil liquids), but only as a relative expression hereof. As, however, it might be of interest to know how much the concentration of hydrogen ions of the extract deviates from that of the natural soil liquid, a series of measurements was made to this effect, as it appeared that it was possible, when the soils were not too dry, by means of a hydraulic press to press a part of the soil liquid out of the soil. Apart from a single exception wood soils were used for these experiments, and it was necessary to take out samples of several kilos in order to have sufficient soil liquid for a measurement of the hydrogen ion concentration. The samples which were brought home in linen bags were severally well mixed; out of each of the great samples a smaller one — 70 cc — was taken out, the hydrogen ion concentration of which was then determined in an extract in the ordinary way. The main samples were then placed one at a time in the press cylinder, the holes of which were so small that the solid particles of the soil could not be pressed through<sup>1)</sup>, and then the soil liquid was pressed out.

In the following table the  $p_{H^+}$  values, electrometrically measured, both of the extracts and of the soil liquid thus pressed out are indicated for a number of soils (the number of the localities

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<sup>1)</sup> At the first experiments the soil was put into a linen bag and was then placed in the cylinder. As the results of the measurements of the hydrogen ion concentration, however, varied according to the bag being used or not, the bag was not used at the final experiment. It must be supposed that certain substances found in the soil liquid were adsorbed by the linen thus altering the hydrogen ion concentration.

refer to the corresponding numbers in the tables 18—26, 31 and 33, where the position of the localities as also their vegetation are stated).

Table 3.

Number of locality	pH <sup>*</sup> of the soil liquid	pH <sup>*</sup> of the extract	Difference (of the extract)
82	3.5	3.6	+ 0.1
88	3.7	3.8	+ 0.1
99	4.0	4.1	+ 0.1
276	4.4	4.8	+ 0.4
149	4.8	5.1	+ 0.3
178	5.1	5.6	+ 0.5
181	5.2	5.6	+ 0.4
219	6.2	6.5	+ 0.3
223	6.2	6.6	+ 0.4
298	7.7	7.7	0
258	7.8	7.6	÷ 0.2

It will be seen from the table that the difference between the pH<sup>\*</sup> value of the soil liquid and that of the extract is not especially great as far as any of the soil samples goes; it is greatest for the soils the pH<sup>\*</sup> value of which lies between 4.3 and 6.3, smallest for the very acid ones. The soil liquid is thus as regards acid soils more acid than the extract of the same soil, while the soil liquid in alkaline soils may be more alkaline than the extract. Plummer (1918) has reached similar results as regards arable soils.

**The buffer action of the soil<sup>1</sup>).** When the hydrogen ion concentration of the soil extract does not deviate more from that of the soil liquid than indicated above, it is of course due to the fact that the soil contains buffer. In order to examine the buffer action of the soil I have determined the hydrogen ion concentration produced in a mixture of soil and water to which gradually is added more acid or base. In fig. 2 the results will be seen of such an experiment made with a humified peaty soil, taken from Jaegersborg Deer-Park, and carrying a rich vegetation

<sup>1</sup> A solution is said to contain buffer if an addition of small quantities of acid or base does not essentially alter its hydrogen ion concentration. See also S. P. L. Sørensen 1909, p. 17 and 48 f. f.

of *Urtica dioeca*. 100 g of the fresh soil were deposited in 200 cc of water; then the  $p_{H^+}$  value of the liquid was electrometrically determined after adding both increasing quantities of 1 n. sulphuric acid and increasing quantities of 1 n. sodium hydroxide. 1 cc was added at a time, and the measurement after each ad-

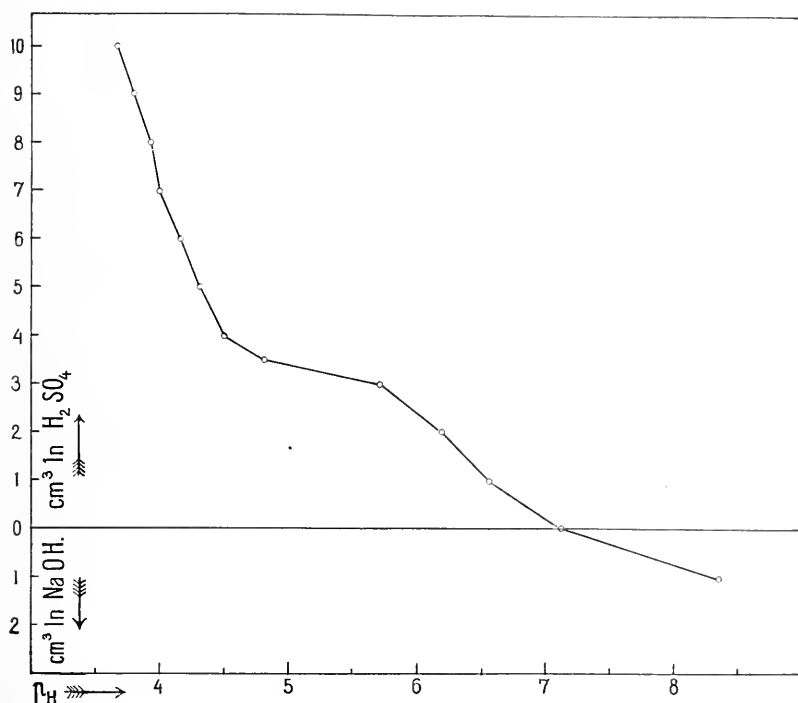


Fig. 2. Titration graph for a peaty soil.

dition was not made until the following day, as the hydrogen ion concentration did not become constant at once. The titration curve reached through the experiment, which consequently expresses the ability of the soil to neutralize acid and base, shows that the soil is in possession of a rather considerable content of buffer. The characteristic flat portions<sup>1)</sup> of the curve over the  $p_{H^+}$  ranges 4.5—6.0 and 7.0—8.0 show that the effect of the buffer in these places is smaller.

<sup>1)</sup> As it might be supposed that the appearance of flat portions of the curve over the  $p_{H^+}$  range 4.5—6.0 might be due to experimental errors, the experiment was repeated with another soil sample. Quite the same result was reached. Also when using hydrochloric acid instead of sulphuric acid the result was the same.

Bjerrum and Gjaldsbæk (1919 p. 63) have in a way similar to that indicated above made titration curves for two soils, i. e. garden soil and a subsoil of a wood bog.

While the soil thus is in possession of rather considerable buffer ability, the filtered soil extracts are quite otherwise. These extracts do not contain any buffer and therefore the addition of even small quantities of acids or bases can alter the concentration of hydrogen ions considerably. Consequently the greatest care must be exercised when measuring the concentration of hydrogen ions of soil extracts.

**How homogeneous and constant is the hydrogen ion concentration of the soil?** In a few localities I have examined the hydrogen ion concentration of the soil in different places within the same homogeneous habitat as regards vegetation (formation). The hydrogen ion concentration is of course not the same in all habitats, where the composition and physiognomy of the vegetation are the same. Within the same homogeneous habitat the different soil samples differed up to 0.5 in  $p_H$  from one another, but in most cases the deviations, however, were smaller. Furthermore it appeared that two soil samples taken from the same spot could deviate reciprocally 0.2 — 0.3 in  $p_H$ . No essential difference appeared whether the soil samples were taken in a depth of 5, 10 or 20 cm., the reciprocal deviations were never greater than 0.3 in  $p_H$ <sup>1</sup>).

Finally samples have been taken out in a few localities with certain intervals during a period of 18 months, in order to examine whether the hydrogen ion concentration varies according to the season. The samples taken out at different times in the same locality might deviate up to 0.5 in  $p_H$  reciprocally, but it was not possible to observe any fixed influence of the season. The concentration of hydrogen ions of the very acid soils, e. g. the raw humus soils, is most constant; the greatest reciprocal deviation between the  $p_H$  values of the samples taken out on the same raw humus locality was 0.2.

**The method used for examining the transformation processes of nitrogen in the soil.** Soil samples of about one kilo

<sup>1</sup> According to Arrhenius (1920) the concentration of hydrogen ions decreases for acid soils with increasing depth. Plummer (1918) on the other hand has observed as far as arable soil is concerned that the hydrogen ion concentration increases with increasing depth.

were taken out for examination. As the degree of moisture of the soil, as will be known, essentially influences the course of the nitrification (Koch 1911), the soil samples were only taken out in periods, in which the soil was neither very moist nor very dry, in order that the results might be compared with one another. 100 g were taken of each sample, which were afterwards dried to constant weight about 18°. The difference between the two weighings is taken as an expression of the percentage content of water in the soil. The remaining parts of the samples were divided into two equal parts; in one half the volume of nitrate and ammonia was determined at once, the other half was transferred into a faïence jar and stood for a month in the Laboratory, covered with a glass sheet, and was then analyzed.

By means of the quantities of water found for the samples the amount of nitrate and ammonia found were passed into and expressed in mg. per one kilo dry soil. By comparing the quantities of nitrate and ammonia found in the samples at once and after one month it will be seen how much nitrogen is transferred during one month in a form accessible for the plants (ammonia, nitrate); thus it is possible to convey an idea of the course of the nitrification and the formation of ammonia in the soil.

*The determination of nitrate.* To determine the amount of nitrate I have used the phenoldisulphonic acid method, a colorimetric method which has been critically treated by Chamot and Pratt (1909, 1910), Stewart and Greaves (1910, 1913), Kelly (1913), Davis (1917), and Noyes (1919 II). The method depends on the following reaction: When the evaporation rest of a nitrate solution is treated with phenoldisulphonic acid, a lemon colour appears after neutralisation with a solution of strong potassium hydroxide; the intensity of this colour is determined by the quantity of nitrate when the phenoldisulphonic acid is found in excess. The yellow compound formed is the tripotassium salt of the nitrophenoldisulphonic acid (Chamot and Pratt 1910) and not, as often said, the potassium salt of the picric acid. The method has been criticized rather much, as some investigators (Kelly 1913, Chamot, Pratt and Redfield 1911) have ascertained loss of nitrate during the analysis. Davis (1917) has, however, shown that these losses are due to the fact that the said investigators have added solution of alum in order to facilitate the filtration of the soil extract, this solution as is well

know reacting like an acid on account of hydrolysis; during the subsequent evaporation of the soil extract nitric acid is lost; this, however, can very easily be prevented, when taking care that the extract reacts alkaline.

At the determination I have followed the following procedure, which has proved to give sufficient exact results: 100 g of fresh soil to which were added 200 cc of water and a small quantity of calciumsulphate (not containing nitrate) were left for an hour during which time it was frequently being stirred; then it was filtered. The addition of calcium sulphate causes that the filtrate quickly will become clear, and that it runs quickly through the filter. 100 cc of the filtrate was evaporated in a porcelain dish on waterbath after adding a small quantity of calciumoxide (about 0.25 g) to a volume of about 10 cc; then it was filtered through a little filter in another dish; the calciumoxide retained all the coloured substances of the extract, consequently the filtrate became completely colourless. The dish was rinsed over the funnel and the filter was washed completely with warm water. This colourless filtrate was evaporated until dryness on waterbath, and the evaporation rest was then treated with 2 cc of phenoldisulphonic acid which by aid of a glass spatula was brought in connection with the whole evaporation rest. After a quarter of an hour 25 cc of water were added and then 10 cc of 10 n potassium hydroxide solution; then the yellow colour appeared. The contents of the dish was then quantitatively transferred into a 200 cc volumetric flask which was made up with water to the mark; 100 cc of this solution were filtered that such precipitate, as generally appears, may be removed, after which the colour-tone of the solution was determined (by means of an ordinary colorimeter) in relation to that of a standard solution prepared in the same way and containing a known quantity of nitrate.

The phenoldisulphonic acid was prepared according to Chalmot, Pratt and Redfield (1911) in the following way: 50 g of pure white phenol were dissolved in 250 cc of pure concentrated sulphuric acid, then 150 cc of fuming sulphuric acid was added (13 per cent  $\text{SO}_3$ ). After being shaken the mixture was heated for two hours on boiling waterbath.

If the phenoldisulphonic acid method is used in the manner just described, it will give correct results within a limit of  $\pm 4$  pct. The determination of the colour-tone of the yellow solution cannot

be done with greater exactitude than  $\pm 4$  per cent, this however being fully sufficient at the present investigations.

I have determined the reliability of the method and the size of the error by adding known quantities of nitrate partly to raw humus soils not containing nitrate, partly to nitrifying soils of which the nitrate has been washed out. I have then analyzed these soils and have recovered the added quantities of nitrate with the said exactitude<sup>1</sup>).

Another fact which I have examined closer is the influence exercised by the time of extraction on the result. A larger soil sample was carefully mixed, then smaller samples of 100 g each were taken out of the former. The quantity of nitrate was then determined in these smaller samples while the time of extraction was varied. It appeared that the above indicated time of extraction, i. e. one hour is sufficient, as more nitrate is never found when a longer time of extraction is used. When a time of extraction of 24 hours is used, as made by Weis and Bondorff (1917) and by the author of this work at former investigations (Olsen 1918), too low quantities of nitrate will be found, especially when the soils analyzed are alkaline. The reason hereof is without doubt the activity of the denitrification bacteria.

*The determination of ammonia.* As is well known, ammonia is highly adsorbed by the soil and consequently only a part of the ammonia found in the soil is found in an aqueous extract of a soil sample. If on the other hand extraction is made with salt solutions, the total quantity of ammonia will be found in the extract (Valmari 1912). The soil samples were, therefore, extracted with 0.5 n sodium chloride solution in the following way: 100 g of soil were shaken for an hour in a shaking machine with 200 cc of 0.5 n sodium chloride solution, after

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<sup>1</sup>) If on the other hand baryta water and not calcium oxide, as mentioned above, was used to remove the coloured substances dissolved in the soil extract, results will be reached which might be subject to very great errors — up to  $\div 40$  per cent; this method has been used by Weis and Bondorff (1917) and by the author of the present work at former investigations (Olsen 1918). This is due to two reasons: firstly a very violent reaction appears when adding phenoldisulphonic acid in case baryta water has been used; hereby nitrogen oxide, which might directly be observed as reddish brown damps, may disappear. Secondly a very great quantity of barium sulphate is formed when adding phenoldisulphonic acid, which incloses a greater or smaller part of the nitrophenoldisulphonic acid.

which the extract was filtered. A certain portion of the filtrate (10—100 cc according to the amount of ammonia found in the extract, as the portion used for the determination must not contain more than 0.8 mg  $\text{NH}_4$ ) was diluted with water till 300 cc and was then distilled with 2 g of magnesia in a Kjeldahl-distilling apparatus; the distillate being gathered in a long-necked 200 cc volumetric flask, containing 5 cc of  $\frac{n}{7}$  hydrochloric acid. The distillation was continued until the distillate filled the flask till the mark. The amount of ammonia was then colorimetrically determined according to Nessler by adding 5 cc of  $\frac{n}{5}$  sodium hydroxide solution and 2 cc potassic mercuric iodide solution. This was made in the manner indicated by Classen (1903 p. 115).

As liquids of comparison in the colorimetric Nessler-determination solution containing known quantities of ammonia sulphate were used, to which were added 5 cc of  $\frac{n}{7}$  hydrochloric acid and was made up with water to 200 cc, then 5 cc of  $\frac{n}{5}$  sodium hydroxide solution and 2 cc potassic mercuric iodide solution were added.

The determination of the colour-tone of the Nessler-analysis in relation to that of the liquids of comparison was made the day after the preparation of the samples by aid of an ordinary colorimeter.

It is a matter of course that water not containing ammonia was used at the preparation of the sodium chloride solution, as also the water used for dilution, filling e. t. c. did not contain ammonia.

At a number of investigations the same soil was extracted both with 0.5 n sodium chloride solution and with distilled water. When analyzing the extracts it appeared that when extracting with water only a quarter of the quantity of ammonia is obtained of that obtained when using 0.5 n sodium chloride solution.

As to the literature on the determination of ammonia in soils, I refer to Baumann (1887), Valmari (1912) and Richmond (1918).

**The determination of the contents of the soil of easily soluble mineral plant nutrients.** The soil of some of the localities examined were extracted with distilled water saturated with carbonic acid, and in this extract the quantity of potassium, phosphate and calcium was determined. Owing to the circum-



stantiality of these analysis it is a very restricted number of localities, the soil of which has been analyzed to determine the said substances; thus no analysis have been made of the soil taken from the meadow localities examined.

The three said plant nutrients have been determined by aid of the method described by Mitscherlich (1907, 1917) one kilo fresh soil being extracted with 5 litres of distilled water. Extraction was made for 12 hours, while the mixture was being frequently stirred which was made mechanically by a glass stirring apparatus worked by an electromotor and by constantly passing carbonic acid through the mixture. The extract thus obtained was filtered according to Mitscherlich through a Chamberland filter.

At the determination of calcium the procedure described by Mitscherlich was closely followed. Furthermore the quantity of potassium was determined according to the method indicated by Mitscherlich and his assistants (Mitscherlich, Celi-chowski and Fischer 1911, Mitscherlich and Fischer 1912; see also Zaleski 1913 and H. Fischer 1914); hereby potassium in precipitated as sodium-potassium cobalti-nitrite, the quantities hereof were determined by titration with potassium permanganate. The analysis were made in close conformity with the procedure indicated by Mitscherlich. The quantity of phosphate was, on the other hand, not determined according to Mitscherlich, but according to the alkalimetric method described by Neumann and Gregersen (Gregersen 1907). I have previously described this procedure (Olsen 1918, p. 38).

The quantity of calcium, potassium and phosphate extracted is in the following given in mg per one kilo dry soil.

### **The hydrogen ion concentration of the meadow soil and the distribution of meadow plants.**

As the object was to study distribution of the meadow plants in relation to a single factor, i. e. the hydrogen ion concentration of the soil, it was expedient that the habitats of the formations examined differed as little as possible as regards other factors. The formations examined therefore are all found on peaty soil, just as they all are found in open places, as such places have been avoided where the shade might be supposed

to affect the vegetation. Many of the meadows examined are, it is true, surrounded by wood, but as these meadows all are very extensive, I have been able to select localities so far from the wood that it was out of the question that the shade might have any effect whatever. None of the localities examined are nearer to the outskirts of the wood than 30 m. Furthermore all the meadows examined are such as are regularly cut. I have avoided meadows that after hay-making are used for cattle-grazing as the cattle-traffic, as far as I have been able to observe, causes a partial alteration of the composition of the vegetation. Finally it is a matter of course that meadows to which have been added manures, or which have been subject to ploughings, sowing or similar cultural intrenchments have been avoided.

The degree of moisture might essentially influence the vegetation and its composition. Therefore it was the original object only to examine meadows, the soils of which were all in the same height over the surface of the subsoil water. This, however, very soon appeared impracticable, as partly the number of meadows suitable for the investigations in consequence hereof would become extremely limited, partly as the researches had to be made at almost the same time, as the level of the subsoil water in the meadows varies very much in the course of the year. Finally it does not quite follow that two meadows the level of the subsoil water of which at a given time lies in the same depth under the surface, also at another time will be lying in the same depth. Therefore it was soon given up only to examine meadows the subsoil water of which was in a certain depth below the surface.

At the final researches localities were included in which the level of the subsoil water at the time of the investigations, i. e. from the beginning of July to the end of September 1920, was found to lie from 30—100 cm below the surface of the meadow. The meadows, which thus were not included in the examinations, were partly the most moist ones, the vegetation of which essentially consists in cyperacees, partly the very dry ones. The level of the subsoil water was observed in ditches and peat pits, of which some always were found in the neighbourhood. I want moreover to observe that the level of the subsoil water in the localities examined hardly have undergone great fluctuations in the period during which the examinations were made.

76 localities have in all been examined of which most are from meadows in the north of Seeland, a few from middle Seeland and from Buderup valley near Skorping in Jutland. Besides the position of the single localities, as also the results of the investigations will appear from the tables 4—12; in these tables the formations examined are arranged according to the hydrogen ion concentration of the soil on the habitats in such a way that the formations from the habitats in the soil of which the lowest  $p_{H^+}$  values are found, are taken first. Each table comprises formations from soils the  $p_{H^+}$  values of which are within a range of 0.5 in  $p_{H^+}$ . Thus 9 tables result, as the  $p_{H^+}$  values found at the analysis of all the soil samples may be grouped in 9  $p_{H^+}$  classes, each comprising a range of 0.5. Each vertical column in the

**Table 4.** Analysis of meadow habitats and their vegetation.

$p_{H^+}$  3.5—3.9.

Locality nos. 1, 2 and 3 are situated in Bure Sea (Grib Forest), no. 4 in Grøftemose (Grib Forest), nos. 5 and 6 in a meadow at the eastern border of Maglemose (Grib Forest) and no. 7 in a meadow in Rold Forest.

$p_{H^+}$ of the soil sample.....	3.8	3.8	3.8	3.8	3.8	3.8	3.9
Number of locality.....	1	2	3	4	5	6	7
<i>Carex Goodenoughii</i> .....	50	»	10	»	»	»	»
<i>Molinia coerulea</i> .....	100	100	100	100	100	60	100
<i>Deschampsia flexuosa</i> .....	100	80	90	100	100	60	70
<i>Sieglingia decumbens</i> .....	»	»	»	»	»	60	10
<i>Agrostis tenuis</i> .....	»	»	»	10	»	50	»
<i>Anthoxanthum odoratum</i> ....	10	»	»	»	»	80	10
<i>Nardus stricta</i> .....	»	»	»	»	»	60	»
<i>Potentilla erecta</i> .....	60	50	70	60	90	60	80
<i>Galium hircynicum</i> .....	100	100	100	60	100	100	100
<i>Rumex acetosa</i> .....	30	40	10	»	20	30	10
<i>Viola palustris</i> .....	»	20	10	»	10	»	10
<i>Peucedanum palustre</i> .....	»	»	»	»	10	10	»
<i>Calluna vulgaris</i> .....	»	»	10	40	10	»	20
<i>Vaccinium vitis idaea</i> .....	»	»	»	30	»	»	10
Number of species.....	7	6	8	7	8	10	10
Density of species.....	4.5	3.9	4.0	4.0	4.4	5.7	4.2
Level of subsoil water in cm	30	50	40	50	40	60	100

Table 5. Analysis of meadow habitats and their vegetation.

PH. 4.0—4.4.

Locality no. 8 is situated in Glarborg Dam (Grib Forest), no. 9 in Rørmose (the northern part of Grib Forest), no. 10 in Sortemose at Farum Sea, nos. 11 and 13 in a meadow in Allindelille Fredskov, nos. 12 and 14 in a meadow south of the halting-place »Slotspavillon« at the Grib Forest railway.

pH of the soil sample.....	4.0	4.1	4.2	4.3	4.4	4.4	4.4
Number of locality.....	8	9	10	11	12	13	14
<i>Eriophorum vaginatum</i> .....	30	30	50	»	»	»	»
<i>Carex Goodenoughii</i> .....	»	»	60	»	»	80	»
— <i>panicea</i> .....	80	»	»	30	»	30	10
<i>Festuca ovina</i> .....	100	»	»	»	»	»	»
<i>Molinia coerulea</i> .....	50	100	40	100	100	100	100
<i>Holcus lanatus</i> .....	»	»	»	»	80	»	20
<i>Deschampsia flexuosa</i> .....	»	80	100	»	50	»	40
<i>Agrostis canina</i> .....	»	»	»	100	»	100	»
— <i>tenuis</i> .....	»	»	»	»	90	»	»
<i>Anthoxanthum odoratum</i> ....	90	»	10	»	»	»	40
<i>Viola palustris</i> .....	10	100	»	60	»	50	»
<i>Potentilla erecta</i> .....	90	100	100	100	100	100	100
<i>Hydrocotyle vulgaris</i> .....	90	»	»	»	»	»	»
<i>Calluna vulgaris</i> .....	»	30	10	100	»	»	»
<i>Oxycoccus quadripetalus</i> ....	»	100	100	10	»	»	»
<i>Galium hircynicum</i> .....	90	100	»	»	»	»	40
<i>Luzula multiflora</i> .....	10	»	»	»	»	»	»
<i>Eriophorum polystachyum</i> ...	»	»	40	»	»	»	»
<i>Carex rostrata</i> .....	10	»	»	»	»	»	»
— <i>sp.</i> .....	»	»	»	»	10	»	»
<i>Festuca rubra</i> .....	»	»	»	»	30	40	40
<i>Salix repens</i> .....	»	»	30	»	10	»	»
<i>Rumex acetosa</i> .....	40	10	»	»	40	»	30
<i>Anemone nemorosa</i> .....	»	»	»	30	»	40	»
<i>Potentilla palustris</i> .....	»	»	20	»	»	»	10
<i>Peucedanum palustre</i> .....	»	»	»	30	»	»	10
<i>Achillea ptarmica</i> .....	»	»	»	»	10	»	10
<i>Hypochaeris radicata</i> .....	»	40	»	»	40	»	»
Number of species.....	12	10	11	9	11	8	12
Density of species.....	6.9	6.9	5.6	5.6	5.6	5.4	4.5
Level of subsoil water in cm	40	40	30	40	50	30	50

**Table 6.** Analysis of meadow habitats and their vegetation.

PH 4.5—4.9.

Locality no. 15 is situated in a meadow in Størgaardsvang (Grib Forest), nos. 16, 18, 20 and 21 in Store Omdemose (Grib Forest), no. 17 in Bure Sea (Grib Forest) and no. 19 in Sortemose at Farum Sea.

pH* of the soil sample.....	4.5	4.6	4.6	4.7	4.7	4.8	4.9
Number of locality.....	15	16	17	18	19	20	21
<i>Carex stellulata</i> .....	»	»	»	»	50	»	»
— <i>Goodenoughii</i> .....	»	»	100	»	»	»	»
— <i>panicea</i> .....	10	»	»	»	100	»	»
<i>Festuca ovina</i> .....	»	»	»	90	40	10	»
— <i>rubra</i> .....	70	20	»	»	»	40	»
<i>Molinia coerulea</i> .....	10	20	10	100	100	50	30
<i>Deschampsia flexuosa</i> .....	»	»	»	50	30	»	»
<i>Sieglingia decumbens</i> .....	»	»	»	»	60	10	»
<i>Agrostis canina</i> .....	»	»	»	»	100	»	»
— <i>tenuis</i> .....	»	»	»	100	»	80	100
<i>Anthoxanthum odoratum</i> ....	100	100	100	30	20	100	100
<i>Salix repens</i> .....	»	»	»	10	60	20	»
<i>Rumex acetosa</i> .....	30	40	80	»	»	»	20
<i>Viola palustris</i> .....	»	»	10	»	80	50	50
<i>Potentilla erecta</i> .....	50	100	50	30	100	60	50
— <i>palustris</i> .....	»	20	10	»	50	10	»
<i>Geum rivale</i> .....	60	»	»	»	»	»	»
<i>Hydrocotyle vulgaris</i> .....	»	»	100	»	80	»	»
<i>Peucedanum palustre</i> .....	»	»	»	»	60	30	»
<i>Veronica officinalis</i> .....	»	»	»	60	»	»	»
<i>Plantago lanceolata</i> .....	60	»	»	30	»	»	»
<i>Campanula rotundifolia</i> ....	»	60	»	40	»	»	20
<i>Galium hircynicum</i> .....	40	»	20	80	»	20	40
<i>Hieracium pilosella</i> .....	»	80	»	20	»	»	»
<i>Carex</i> sp.....	»	»	»	»	10	»	»
<i>Holcus lanatus</i> .....	20	20	30	30	10	20	»
<i>Rumex acetosella</i> .....	20	»	»	10	»	»	»
<i>Ranunculus acer</i> .....	20	10	»	»	»	10	»
<i>Hypericum perforatum</i> .....	»	»	»	10	»	»	»
<i>Calluna vulgaris</i> .....	»	10	10	»	»	10	10
<i>Menyanthes trifoliata</i> .....	»	»	»	»	10	»	»
<i>Succisa pratensis</i> .....	»	»	»	»	10	»	»
<i>Hypochoeris radicata</i> .....	»	»	»	10	»	»	10
Number of species.....	12	11	11	16	18	15	10
Density of species.....	4.9	4.8	5.2	7.0	9.7	5.2	4.3
Level of subsoil water in cm	50	80	40	35	30	70	70

Table 7. Analysis of meadow habitats and their vegetation.

pH: 5.0—5.4.

Locality nos. 22, 24 and 26 are situated in Sortemose at Farum Sea, no. 23 in Buderup valley at Skorping, no. 25 in a meadow south of the halting-place "Slotspavillonen" at the Grib Forest railway, no. 27 in the large meadow in Stenholt enclosure (Grib Forest), no. 28 in Store Ondemose in Stregaardsvang (Grib Forest) and no. 29 in a meadow, north-east of Selbæk Mose (Grib Forest).

pair of the soil sample	5.0	5.0	5.1	5.1	5.3	5.3	5.3	5.4
Number of locality	22	23	24	25	26	27	28	29
<i>Luzula multiflora</i> .....	20	30	60	»	»	60	»	»
<i>Carex stellulata</i> .....	50	»	»	»	»	»	»	»
— <i>Goodenoughii</i> .....	10	100	»	»	»	»	»	»
— <i>panicæ</i> .....	80	10	30	10	»	10	»	10
— <i>lasiocarpa</i> .....	»	»	»	»	100	»	»	»
<i>Festuca rubra</i> .....	80	30	50	100	80	100	100	30
<i>Poa pratensis</i> .....	40	»	»	80	»	70	»	»
<i>Holcus lanatus</i> .....	40	»	80	100	70	80	90	»
<i>Sieglingia decumbens</i> .....	50	40	»	»	»	»	»	»
<i>Calamagrostis lanceolata</i> .....	»	»	»	»	60	»	»	»
<i>Agrostis canina</i> .....	»	70	100	20	100	»	»	»
— <i>tenuis</i> .....	100	»	»	40	»	40	100	»
<i>Anthoxanthum odoratum</i> .....	100	90	100	30	20	100	100	100
<i>Salix repens</i> .....	»	»	»	20	20	»	90	»
<i>Rumex acetosa</i> .....	»	»	20	50	»	50	10	30
<i>Ranunculus acris</i> .....	»	»	»	30	»	40	»	90
<i>Viola palustris</i> .....	100	100	80	10	40	»	»	»
<i>Potentilla erecta</i> .....	100	100	100	20	100	40	80	40
— <i>palustris</i> .....	10	40	70	10	100	30	»	30
<i>Geum rivale</i> .....	»	»	60	»	»	50	»	100
<i>Hydrocotyle vulgaris</i> .....	70	»	20	»	70	»	»	»



Table 8. Analysis of meadow habitats and their vegetation.

pH: 5.5—5.9.

Locality nos. 30 and 36 are situated in Sortemose at Farum Sea, no. 31 in a meadow in Stregardsvang (Grib Forest), nos. 32 and 33 in Selbak Mose in Tibberup Holme (Grib Forest), no. 34 in Tokkerup Enghave (Grib Forest), nos. 35, 37 and 43 in Store Omdemose in Stregardsvang (Grib Forest), no. 38 in Bure Sea (Grib Forest), no. 39 in Buderup valley at Skorping, nos. 40 and 42 in the large meadow in Stenholt enclosure (Grib Forest) and no. 41 in Lille Hæsemose (northern part of Grib Forest).

pair of the soil sample .....	5.5	5.5	5.5	5.5	5.5	5.5	5.6	5.6	5.6	5.7	5.7	5.7	5.8	5.9
Number of locality .....	30	31	32	33	34	35	36	37	38	39	40	41	42	43
<i>Carex pulicaris</i> .....	70	»	»	»	»	»	»	»	»	60	»	»	»	»
— <i>Goodenoughii</i> .....	10	40	»	»	»	»	100	»	»	100	»	»	»	»
— <i>panicea</i> .....	»	»	»	»	»	»	60	»	»	100	»	»	»	»
<i>Dactylis glomerata</i> .....	100	100	100	100	90	10	100	70	100	100	100	100	100	100
<i>Festuca rubra</i> .....	»	60	60	»	60	10	»	40	»	»	60	40	100	100
<i>Poa pratensis</i> .....	100	70	50	80	20	70	70	100	30	60	100	»	100	100
<i>Holcus lanatus</i> .....	100	10	»	30	100	100	»	20	100	»	»	10	100	100
<i>Deschampsia caespitosa</i> .....	»	»	»	»	»	»	»	»	»	70	»	»	»	»
<i>Sieglingia decumbens</i> .....	»	»	»	80	»	»	»	»	»	»	»	»	»	»
<i>Calamagrostis lanceolata</i> .....	»	»	10	»	»	»	100	»	»	70	»	»	»	»
<i>Agrostis canina</i> .....	70	»	»	»	»	»	»	»	»	»	»	»	»	»
— <i>tenuis</i> .....	»	»	40	»	50	20	100	40	90	»	»	»	»	»
<i>Anthoxanthum odoratum</i> .....	100	100	100	100	»	40	100	60	60	100	100	100	40	»
<i>Rumex acetosa</i> .....	40	50	90	20	20	10	10	10	100	»	100	50	10	80
<i>Ranunculus acer</i> .....	100	100	80	70	30	10	100	40	100	20	80	»	10	»
<i>Viola palustris</i> .....	»	»	»	»	»	10	60	70	»	100	»	»	»	»
<i>Potentilla erecta</i> .....	50	»	»	»	»	20	80	»	»	40	»	»	»	»
— <i>palustris</i> .....	60	»	»	»	»	»	50	»	»	80	»	»	»	»
<i>Geum rivale</i> .....	60	70	100	90	30	30	100	100	100	»	100	100	100	50
<i>Veronica chamaedrys</i> .....	40	»	»	30	»	»	»	»	10	»	»	»	»	50
<i>Plantago lanceolata</i> .....	20	100	60	20	»	»	40	100	»	»	»	90	»	»
<i>Brucella vulgaris</i> .....	»	»	»	»	»	»	»	10	»	60	»	»	»	»





Table 9. Analysis of meadow

pH 6.

Locality nos. 44 and 53 are situated in Kormose between Roskilde and Boserup wood, nos. 45 and 51 in the part of Grib Forest, no. 48 in Lerbæks Rende in Store Bogeskov at Gyrstinge, nos. 49 and 52 in the part of Grib Forest.

Loc. of the soil sample.....	6.0	6.0	6.0	6.0	6.2	6.2	6.2	6.2	6.2	6.3	6.3
Number of locality.....	44	45	46	47	48	49	50	51	52	53	54
<i>Carex paradoxa</i> .....	3	3	3	3	3	3	3	3	3	50	3
— <i>Goodenoughii</i> .....	3	10	80	3	3	70	100	3	3	3	3
— <i>panicea</i> .....	3	3	3	3	3	70	10	3	3	90	3
<i>Dactylis glomerata</i> .....	70	3	3	3	3	3	3	3	3	3	3
<i>Festuca rubra</i> .....	60	100	100	100	100	100	60	100	100	30	100
— <i>elatior</i> .....	70	3	3	3	3	3	3	3	3	3	3
<i>Poa pratensis</i> .....	80	50	3	80	20	3	40	50	100	3	100
<i>Briza media</i> .....	3	3	3	3	3	70	30	3	3	3	3
<i>Phragmites communis</i> .....	3	3	3	3	3	40	3	3	3	50	3
<i>Molinia coerulea</i> .....	3	3	3	3	3	3	3	3	3	50	3
<i>Holcus lanatus</i> .....	60	50	100	3	10	60	70	60	3	3	40
<i>Deschampsia caespitosa</i> ....	90	20	80	50	70	3	3	20	100	30	100
<i>Avena pubescens</i> .....	10	3	3	3	3	3	3	50	3	3	3
<i>Agrostis canina</i> .....	3	3	100	3	3	3	3	3	3	3	3
<i>Cynosurus cristatus</i> .....	3	3	3	3	3	50	3	3	3	3	3
<i>Anthoxanthum odoratum</i> ...	3	70	80	100	70	100	100	50	40	3	3
<i>Rumex acetosa</i> .....	70	20	80	90	3	50	40	100	50	3	40
<i>Ranunculus acer</i> .....	70	50	90	3	40	50	100	40	40	30	70
<i>Cardamine pratensis</i> .....	3	60	3	3	3	20	3	3	3	3	3
<i>Parnassia palustris</i> .....	3	3	3	3	3	3	30	3	3	60	3
<i>Filipendula ulmaria</i> .....	30	3	3	40	60	3	20	3	3	10	80
<i>Potentilla erecta</i> .....	3	3	3	3	3	40	3	3	3	50	3
— <i>reptans</i> .....	3	3	3	3	3	3	70	3	3	3	3
— <i>anserina</i> .....	3	3	3	3	3	3	60	3	3	3	3
— <i>palustris</i> .....	3	30	3	10	3	3	3	50	3	3	3
<i>Geum rivale</i> .....	90	3	3	70	100	100	100	50	100	3	100
<i>Lotus corniculatus</i> .....	3	3	3	3	3	70	100	3	3	3	3
<i>Hydrocotyle vulgaris</i> .....	3	3	70	3	3	3	3	3	3	3	3
<i>Veronica chamaedrys</i> .....	3	3	3	3	20	3	3	3	3	3	60
<i>Plantago lanceolata</i> .....	3	3	70	3	3	100	100	3	30	3	100
<i>Brunella vulgaris</i> .....	3	3	20	3	10	90	3	3	3	70	3
<i>Menta aquatica</i> .....	3	3	3	3	3	3	3	3	3	50	3
<i>Galium palustre</i> .....	10	10	3	30	100	70	100	50	3	10	50
<i>Succisa pratensis</i> .....	3	3	3	3	3	3	3	3	3	50	3
<i>Cirsium palustre</i> .....	3	3	3	3	40	20	70	3	3	40	3

habitats and their vegetation.

-6.4.

the large meadow in Stenholt enclosure (Grib Forest), nos. 47 and 52 in Lille Hessemose (northern Buderup valley at Skorping, nos. 46 and 54 in a meadow in Strogaardsvang (Grib Forest).

ph. of the soil sample....	6.0	6.0	6.0	6.0	6.2	6.2	6.2	6.2	6.2	6.3	6.3
Number of locality.....	44	45	46	47	48	49	50	51	52	53	54
<i>Equisetum arvense</i> .....	»	»	»	»	20	»	»	»	»	»	»
<i>Luzula multiflora</i> .....	»	»	»	»	»	»	40	»	»	»	»
<i>Carex pulicaris</i> .....	»	»	»	»	»	»	»	»	»	20	»
— <i>glauca</i> .....	»	»	»	»	10	»	»	»	»	30	»
— <i>rostrata</i> .....	»	»	»	»	»	»	20	»	»	»	»
— <i>sp.</i> .....	»	»	»	»	»	»	»	»	»	20	»
<i>Festuca ovina</i> .....	30	»	»	»	»	10	»	»	»	»	»
<i>Avena pratensis</i> .....	»	»	»	»	»	»	»	»	»	»	10
<i>Salix repens</i> .....	»	»	»	»	»	»	»	»	»	20	»
<i>Lychnis flos-cuculi</i> .....	»	»	10	»	»	»	10	»	»	»	»
<i>Ranunculus flammula</i> .....	»	»	10	»	»	»	»	»	»	»	»
— <i>repens</i> .....	»	»	»	»	20	»	»	»	»	»	»
<i>Viola palustris</i> .....	»	»	»	»	10	40	10	»	»	»	»
<i>Lathyrus pratensis</i> .....	30	»	»	»	40	»	»	40	»	»	»
<i>Trifolium pratense</i> .....	»	»	»	»	»	30	»	»	»	»	»
<i>Angelica silvestris</i> .....	»	»	»	»	»	»	30	»	»	40	»
<i>Veronica scutellata</i> var. <i>villosa</i> .....	»	»	»	»	20	»	»	»	»	»	»
<i>Euphrasia curta</i> .....	»	»	»	»	»	10	»	»	»	»	»
<i>Alectorolophus major</i> .....	»	»	»	»	»	»	10	»	»	»	»
<i>Pinguicula vulgaris</i> .....	»	»	»	»	»	10	»	»	»	»	»
<i>Plantago lanceolata</i> var. <i>dubia</i> .....	»	»	»	»	»	»	»	»	»	»	10
<i>Ajuga reptans</i> .....	»	»	»	»	10	»	»	»	»	»	»
<i>Galium uliginosum</i> .....	»	»	»	»	»	»	»	40	»	»	»
— <i>boreale</i> .....	»	»	»	»	»	»	»	»	»	40	»
<i>Valeriana dioeca</i> .....	»	»	»	»	10	»	»	»	»	»	»
<i>Tussilago farfarus</i> .....	»	»	»	»	»	»	10	»	»	»	»
<i>Achillea millefolium</i> .....	»	»	»	»	»	»	10	»	»	»	»
<i>Hieracium pilosella</i> .....	»	»	»	»	10	30	»	»	»	»	»
<i>Crepis praemorsa</i> .....	»	»	»	»	»	»	»	»	»	10	»
<i>Taraxacum sp.</i> .....	»	»	»	»	»	»	»	»	»	20	»
Number of species .....	14	11	13	9	21	24	26	13	8	23	13
Density of species .....	7.7	4.7	8.9	5.7	7.9	13.0	13.4	7.0	5.6	8.7	8.6
Level of subsoil water in cm	60	100	30	70	100	40	40	100	70	50	100

Table 10. Analysis of meadow

PH. 6.5

Locality no. 55 is situated in Lyngby Mose, no. 56 in Rørmose between Roskilde and Bøse  
in Stenholt enclosure, nos. 59 and 62 i Selbæk Mose in Tibberup

pH. of the soil sample .....	6.5	6.5	6.6	6.7	6.7	6.8	6.8	6.8
Number of locality .....	55	56	57	58	59	60	61	62
<i>Scirpus silvaticus</i> .....	60	»	»	30	»	20	100	40
<i>Carex rostrata</i> .....	30	»	100	»	»	»	»	»
<i>Dactylis glomerata</i> .....	50	100	»	20	»	»	»	60
<i>Festuca ovina</i> .....	»	50	»	»	»	»	»	»
— <i>rubra</i> .....	100	70	100	»	100	100	100	100
— <i>elatior</i> .....	20	40	»	90	100	100	60	20
<i>Poa pratensis</i> .....	50	30	»	»	60	30	80	30
<i>Briza media</i> .....	»	»	»	20	»	70	»	»
<i>Holcus lanatus</i> .....	100	40	100	70	20	100	100	60
<i>Deschampsia caespitosa</i> .....	»	40	»	10	»	»	70	10
<i>Avena elatior</i> .....	»	»	»	»	40	»	»	60
<i>Alopecurus pratensis</i> .....	»	»	»	»	100	»	»	60
<i>Rumex acetosa</i> .....	20	30	100	20	100	20	»	20
<i>Trollius europaeus</i> .....	»	10	»	»	»	»	80	»
<i>Ranunculus acer</i> .....	20	60	100	10	50	70	40	60
<i>Linum catharticum</i> .....	»	»	»	»	60	»	»	»
<i>Filipendula ulmaria</i> .....	80	30	30	70	40	50	50	50
<i>Geum rivale</i> .....	100	100	100	30	40	20	»	100
<i>Lathyrus pratensis</i> .....	»	»	»	»	»	70	40	»
<i>Angelica silvestris</i> .....	50	20	»	»	20	»	»	40
<i>Veronica chamaedrys</i> .....	»	»	»	10	10	60	10	40
<i>Plantago lanceolata</i> .....	»	30	100	»	20	40	30	100
<i>Valeriana dioeca</i> .....	60	»	»	»	»	»	»	»
<i>Cirsium oleraceum</i> .....	80	30	»	100	»	80	100	100
<i>Equisetum palustre</i> .....	»	»	»	»	»	»	10	»
<i>Juncus lamprocarpus</i> .....	»	»	»	»	»	10	»	»
<i>Carex elata</i> .....	»	»	»	»	»	10	»	»
— <i>Goodenoughii</i> .....	»	»	10	»	»	»	»	»
— <i>glaucia</i> .....	»	»	»	»	»	40	»	»
— <i>panicca</i> .....	»	»	20	»	»	10	»	»
— <i>acutiformis</i> .....	40	»	»	»	»	»	»	»
— <i>hirta</i> .....	»	»	»	20	»	»	10	»

habitats and their vegetation.

-6.9.

up wood, no. 57 in Grønnekilde meadow (Grib Forest), nos. 58 and 61 in the large meadow  
olme (Grib Forest) and no. 60 in Sortemose at Farum Sea.

pH <sup>r</sup> of the soil sample .....	6.5	6.5	6.6	6.7	6.7	6.8	6.8	6.8
Number of locality .....	55	56	57	58	59	60	61	62
<i>Carex</i> sp. ....	»	»	10	»	»	»	»	30
<i>Phragmites communis</i> .....	»	»	40	»	»	30	»	»
<i>Molinia coerulea</i> .....	»	40	»	»	»	»	»	»
<i>Avena pratensis</i> .....	»	»	»	»	20	»	»	»
— <i>pubescens</i> .....	»	10	»	40	»	»	40	»
<i>Trisetum flavescens</i> .....	»	»	»	»	10	»	»	40
<i>Agrostis alba</i> .....	»	»	»	»	»	30	»	»
<i>Anthoxanthum odoratum</i> .....	»	20	40	»	20	»	»	»
<i>Polygonum amphibium</i> .....	»	»	»	»	»	10	»	»
<i>Parnassia palustris</i> .....	10	»	»	»	»	»	»	»
<i>Potentilla erecta</i> .....	»	10	»	»	»	»	»	»
— <i>palustris</i> .....	»	»	20	»	»	»	»	»
<i>Vicia cracca</i> .....	»	»	»	»	»	10	»	»
<i>Trifolium pratense</i> .....	»	»	»	»	»	40	»	»
<i>Epilobium palustre</i> .....	40	»	40	»	»	»	»	»
<i>Carum carvi</i> .....	»	20	»	»	»	»	»	»
<i>Anthriscus silvester</i> .....	»	20	»	»	»	»	»	10
<i>Primula veris</i> .....	»	10	»	»	»	»	»	»
<i>Brunella vulgaris</i> .....	40	40	»	»	»	40	»	»
<i>Nepeta glechoma</i> .....	»	»	»	20	»	»	»	10
<i>Menta aquatica</i> .....	»	40	»	»	»	»	40	»
<i>Galium palustre</i> .....	»	20	10	»	40	40	»	»
<i>Succisa pratensis</i> .....	»	20	»	»	»	30	»	»
<i>Cirsium palustre</i> .....	20	»	40	10	»	40	»	10
<i>Tussilago farfara</i> .....	»	»	»	»	10	»	»	»
<i>Achillea ptarmica</i> .....	»	20	»	»	»	»	10	»
— <i>millefolium</i> .....	»	»	»	»	»	10	»	»
<i>Taraxacum</i> sp. ....	»	10	»	»	»	»	10	»
Number of species .....	19	28	17	16	19	28	19	22
Density of species .....	9.7	9.6	9.6	5.7	8.6	11.8	9.8	10.5
Level of subsoil water in cm .....	40	50	30	100	100	30	100	100

Table 11. Analysis of meadow

pH: 7.6

Locality no. 63 is situated in Buderup valley at Skorping, nos. 64, 65, 66 and 67 in Rørmose  
in a meadow south of Kong

Loc. of the soil sample.....	7.0	7.0	7.0	7.1	7.3	7.3	7.3	7.4
Number of locality.....	63	64	65	66	67	68	69	70
<i>Scirpus silvaticus</i> .....	»	»	»	»	»	»	»	100
<i>Carex paradoxa</i> .....	»	»	»	50	»	»	»	»
— <i>Goodenoughii</i> .....	100	»	»	40	»	»	»	»
— <i>glauca</i> .....	30	100	»	60	20	50	»	»
— <i>panicea</i> .....	»	»	»	80	»	40	»	»
<i>Dactylis glomerata</i> .....	»	20	»	»	100	»	100	100
<i>Festuca rubra</i> .....	70	100	80	90	80	100	»	80
— <i>elatior</i> .....	»	100	100	»	100	100	20	100
<i>Poa pratensis</i> .....	»	»	»	»	20	60	60	70
<i>Briza media</i> .....	10	»	»	40	»	70	»	70
<i>Phragmites communis</i> .....	20	40	20	60	»	»	10	20
<i>Molinia coerulea</i> .....	»	»	»	50	»	»	»	10
<i>Holcus lanatus</i> .....	100	30	60	»	70	»	50	80
<i>Deschampsia caespitosa</i> .....	»	40	»	100	20	50	»	50
<i>Avena pubescens</i> .....	»	»	»	»	»	70	»	80
— <i>elatior</i> .....	»	»	»	»	»	»	100	»
<i>Agrostis alba</i> .....	»	»	80	»	50	»	»	»
<i>Rumex acetosa</i> .....	100	»	20	»	30	»	»	»
<i>Ranunculus acer</i> .....	100	90	70	»	90	100	»	90
<i>Linum catharticum</i> .....	»	»	»	»	»	»	»	70
<i>Parnassia palustris</i> .....	»	50	»	»	»	»	»	»
<i>Filipendula ulmaria</i> .....	»	10	»	50	»	»	»	»
<i>Potentilla anserina</i> .....	»	»	70	»	»	»	»	»
<i>Geum rivale</i> .....	100	50	»	10	30	90	»	40
<i>Vicia cracca</i> .....	»	50	»	»	»	»	»	»
<i>Lathyrus pratensis</i> .....	100	100	»	20	»	»	»	»
<i>Trifolium pratense</i> .....	100	10	»	»	»	»	»	»
<i>Angelica silvestris</i> .....	»	50	80	»	»	20	»	40
<i>Convolvulus sepium</i> .....	»	»	»	»	»	»	100	»
<i>Euphrasia tenuis</i> .....	50	»	»	»	»	»	»	»
<i>Plantago lanceolata</i> .....	100	50	10	»	»	80	»	10
<i>Brunella vulgaris</i> .....	80	20	»	100	»	70	»	»
<i>Mentha aquatica</i> .....	»	»	40	»	30	»	»	100
<i>Galium palustre</i> .....	»	10	»	»	»	40	»	60
<i>Succisa pratensis</i> .....	10	80	»	10	»	60	»	10

habitats and their vegetation.

7.4.

between Roskilde and Boserup wood, nos. 68 and 70 in Sortemose at Farum Sea and no. 69  
at Jægersborg deer-park.

pH of the soil sample.....	7.0	7.0	7.0	7.1	7.3	7.3	7.3	7.4
Number of locality.....	63	64	65	66	67	68	69	70
<i>Cirsium oleraceum</i> .....	»	100	100	60	100	100	100	100
— <i>palustre</i> .....	50	»	»	30	»	»	10	»
<i>Tussilago farfara</i> .....	40	»	50	»	80	»	50	»
<i>Taraxacum</i> sp. ....	»	»	60	»	20	»	»	40
<i>Equisetum fluviatile</i> .....	10	30	10	»	»	»	»	»
<i>Juncus lampocarpus</i> .....	10	30	»	»	10	»	»	»
<i>Triglochin maritima</i> .....	20	»	»	»	»	»	»	»
<i>Carex hirta</i> .....	10	»	»	»	»	»	»	20
<i>Anthoxanthum odoratum</i> .....	»	»	»	»	»	30	»	»
<i>Orchis latifolia</i> .....	»	»	»	»	»	»	»	10
<i>Helleborine palustris</i> .....	»	»	»	10	»	»	»	»
<i>Cerastium caespitosum</i> .....	10	»	»	»	10	»	»	»
<i>Trollius europaeus</i> .....	»	30	30	»	»	»	»	20
<i>Hypericum maculatum</i> .....	»	»	»	20	»	»	»	»
<i>Potentilla erecta</i> .....	»	10	»	40	»	»	»	10
— <i>reptans</i> .....	»	»	»	20	»	»	»	»
<i>Trifolium repens</i> .....	20	»	»	»	»	»	»	»
<i>Lotus corniculatus</i> .....	20	»	»	»	»	»	»	»
<i>Anthriscus silvester</i> .....	»	»	»	»	20	»	»	»
<i>Peucedanum palustre</i> .....	»	»	»	»	10	»	»	»
<i>Lysimachia vulgaris</i> .....	»	»	»	10	»	»	»	»
<i>Veronica chamaedrys</i> .....	»	»	»	»	»	10	»	»
<i>Plantago major</i> .....	30	»	10	»	»	30	»	»
<i>Nepeta glechoma</i> .....	»	»	10	»	»	»	»	»
<i>Galium uliginosum</i> .....	»	20	»	»	»	»	»	»
<i>Centaurea jacea</i> .....	10	»	»	»	»	»	»	»
<i>Eupatorium cannabinum</i> .....	»	»	»	»	»	»	»	10
<i>Achillea ptarmica</i> .....	»	»	»	»	»	10	»	»
— <i>millefolium</i> .....	10	20	»	»	»	»	»	»
<i>Inula salicina</i> .....	»	»	»	»	20	»	»	»
<i>Crepis praemorsa</i> .....	»	»	»	10	»	»	»	»
Number of species .....	27	26	18	22	20	20	10	26
Density of species .....	13.1	12.4	9.0	9.6	9.1	11.8	6.0	13.9
Level of subsoil water in cm.	40	50	60	40	70	50	60	30

Table 12. Analysis of meado

PH. 7.

Locality no. 71 is situated in Rormose between Roskilde and Boserup wood, no. 72 in Sorpark and no. 76 in a meadow south

part of the soil sample.....	7.5	7.5	7.5	7.5	7.6	7.7
Number of locality.....	71	72	73	74	75	76
<i>Scirpus silvaticus</i> .....	»	60	»	»	»	100
<i>Carex Goodenoughii</i> .....	»	60	»	»	»	»
— <i>glauca</i> .....	50	60	»	»	»	»
— <i>acutiformis</i> .....	»	»	»	»	»	70
— <i>hirta</i> .....	»	30	80	20	»	10
<i>Dactylis glomerata</i> .....	80	»	70	60	40	»
<i>Festuca rubra</i> .....	100	100	100	»	100	100
— <i>elatior</i> .....	100	100	40	»	»	50
<i>Poa pratensis</i> .....	10	50	30	10	»	30
<i>Briza media</i> .....	»	100	»	»	»	»
<i>Phragmites communis</i> .....	50	10	»	60	»	10
<i>Holcus lanatus</i> .....	30	10	100	30	10	70
<i>Avena pubescens</i> .....	»	100	»	»	»	»
— <i>elatior</i> .....	»	»	50	30	»	»
<i>Agrostis alba</i> .....	20	»	20	100	100	»
<i>Phleum pratense</i> .....	50	»	»	»	»	10
<i>Ranunculus acer</i> .....	80	100	10	»	»	»
<i>Linum catharticum</i> .....	»	100	»	»	»	»
<i>Potentilla reptans</i> .....	10	»	»	70	»	»
— <i>anserina</i> .....	50	»	»	70	»	50
<i>Lathyrus pratensis</i> .....	»	»	»	»	»	50
<i>Nepeta glechoma</i> .....	»	»	»	60	»	30
<i>Menta aquatica</i> .....	10	20	10	100	»	»
<i>Cirsium oleraceum</i> .....	100	100	100	90	80	70
<i>Tussilago farfara</i> .....	»	»	90	80	»	70
<i>Taraxacum</i> sp. ....	100	»	10	»	»	»
<i>Equisetum palustre</i> .....	40	40	20	»	»	»
<i>Luzula multiflora</i> .....	»	10	»	»	»	»



habitats and their vegetation.

-7.9.

ose at Farum Sea, nos. 73, 74 and 75 in a meadow south of Kongeport at Jægersborg deer-odeport at Jægersborg deer-park.

pH <sup>+</sup> of the soil sample .....	7.5	7.5	7.5	7.5	7.6	7.7
Number of locality .....	71	72	73	74	75	76
<i>Carex panicea</i> .....	»	40	»	»	»	»
<i>Glyceria aquatica</i> .....	»	»	»	10	»	»
<i>Deschampsia caespitosa</i> .....	20	40	»	»	10	20
<i>Avena pratensis</i> .....	»	»	10	»	»	»
<i>Anthoxanthum odoratum</i> .....	»	30	»	»	»	»
<i>Rumex hydrolapathum</i> .....	»	»	»	»	10	»
<i>Polygonum amphibium</i> .....	»	»	»	»	10	»
<i>Trollius europaeus</i> .....	40	»	»	»	»	»
<i>Hypericum acutum</i> .....	»	»	»	»	»	10
<i>Geum rivale</i> .....	10	40	»	»	»	»
<i>Alchemilla alpestris</i> .....	»	»	»	20	»	»
<i>Vicia cracca</i> .....	»	10	»	»	»	»
<i>Trifolium procumbens</i> .....	»	10	»	»	»	»
— <i>pratense</i> .....	»	20	»	»	20	»
<i>Medicago lupulina</i> .....	»	40	»	»	»	»
<i>Lythrum salicaria</i> .....	»	»	»	»	10	»
<i>Anthriscus silvester</i> .....	»	»	»	»	20	»
<i>Angelica silvestris</i> .....	40	»	30	30	»	20
<i>Convolvulus sepium</i> .....	»	»	»	30	20	»
<i>Veronica chamaedrys</i> .....	»	10	»	»	»	»
<i>Pedicularis palustris</i> .....	»	10	»	»	»	»
<i>Plantago lanceolata</i> .....	20	40	»	»	»	»
<i>Galium palustre</i> .....	»	»	»	»	»	10
<i>Cirsium arvense</i> .....	»	»	»	»	»	20
Number of species .....	21	28	16	17	12	19
Density of species .....	10.1	13.4	7.7	8.7	4.3	8.0
Level of subsoil water in cm .....	50	30	70	40	50	70



## habitats and their vegetation.

with alkaline peaty soil (locality nos. 72 and 70) over a distance of a few hundred metres. Nitrogen ion concentration of the peat in the direction of the high moor and the consequent analysis mentioned here also are stated, apart from localities nos. 77 and 78 (high moor localities), as in all the localities 30 cm.

pH of the soil sample....	7.5	7.4	6.8	5.6	5.5	5.3	5.1	4.7	4.2	3.8	3.6
Number of locality.....	72	70	60	36	30	26	24	19	10	77	78
<i>Calluna vulgaris</i> .....	»	»	»	»	»	»	»	»	10	40	80
<i>Andromeda polifolia</i> .....	»	»	»	»	»	»	»	»	»	10	50
<i>Equisetum palustre</i> .....	40	»	»	»	»	»	»	»	»	»	»
<i>Juncus lampocarpus</i> .....	»	»	10	»	»	»	»	»	»	»	»
<i>Eriophorum polystachyum</i> ..	»	»	»	10	»	»	»	»	40	»	»
<i>Carex elata</i> .....	»	»	10	»	»	»	»	»	»	»	»
— <i>rostrata</i> .....	»	»	»	»	»	10	»	»	»	»	»
— <i>hirta</i> .....	30	20	»	»	»	»	»	»	»	»	»
— <i>sp.</i> .....	»	»	»	10	»	»	»	10	»	»	»
<i>Festuca ovina</i> .....	»	»	»	30	10	»	40	40	»	»	»
<i>Phragmites communis</i> .....	10	20	30	»	»	»	»	»	»	»	»
<i>Agrostis alba</i> .....	»	»	30	»	»	»	»	»	»	»	»
<i>Orchis latifolius</i> .....	»	10	»	»	»	»	»	»	»	»	»
<i>Rumex acetosa</i> .....	»	»	20	»	40	»	20	»	»	»	»
<i>Polygonum amphibium</i> ....	»	»	10	»	»	»	»	»	»	»	»
<i>Lychnis flos-cuculi</i> .....	»	»	»	»	»	»	10	»	»	»	»
<i>Trollius europaeus</i> .....	»	20	»	»	»	»	»	»	»	»	»
<i>Vicia cracca</i> .....	10	»	10	30	»	»	»	»	»	»	»
<i>Trifolium procumbens</i> ....	10	»	»	»	»	»	»	»	»	»	»
— <i>pratense</i> .....	20	»	40	»	»	»	»	»	»	»	»
<i>Medicago lupulina</i> .....	40	»	»	»	»	»	»	»	»	»	»
<i>Angelica silvestris</i> .....	»	40	»	»	»	»	»	»	»	»	»
<i>Erica tetralix</i> .....	»	»	»	»	»	»	»	»	»	»	10
<i>Pedicularis palustris</i> .....	10	»	»	»	»	»	»	»	»	»	»
<i>Plantago lanceolata</i> .....	40	10	40	»	20	»	»	»	»	»	»
<i>Brunella vulgaris</i> .....	»	»	40	40	»	10	»	»	»	»	»
<i>Scutellaria galericulata</i> ....	»	»	»	»	40	»	»	»	»	»	»
<i>Menyanthes trifoliata</i> .....	»	»	»	»	»	»	»	10	»	»	»
<i>Valeriana sp.</i> .....	»	»	»	»	»	10	»	»	»	»	»
<i>Succisa pratensis</i> .....	»	10	30	10	»	»	»	10	»	»	»
<i>Cirsium palustre</i> .....	»	»	40	20	10	30	40	»	»	»	»
<i>Eupatorium cannabinum</i> ...	»	10	»	»	»	»	»	»	»	»	»
<i>Achillea millefolium</i> .....	»	»	10	»	»	»	»	»	»	»	»
<i>Taraxacum sp.</i> .....	»	40	»	»	»	»	»	»	»	»	»
Number of species .....	28	26	28	23	20	18	17	18	11	7	7
Density of species .....	13.4	13.9	11.8	11.1	10.5	8.8	8.8	9.7	5.6	3.4	3.9

Table 14. The average frequency of the meadow plants in the pH<sub>r</sub> classes.

pH <sub>r</sub> classes	3.5-3.9	4.0-4.4	4.5-4.9	5.0-5.4	5.5-5.9	6.0-6.4	6.5-6.9	7.0-7.4	7.5-7.9	Occurrence on number of localities
<i>Deschampsia flexuosa</i>	86	68	40		»	»	»	»	»	13
<i>Galium harenicum</i>	94	77	40	20	15	»	»	»	»	18
<i>Molinia coerulea</i> ...	94	84	46	13	»	50	40	30	»	28
<i>Potentilla erecta</i> ....	67	99	63	73	48	45	10	20	»	39
<i>Calluna vulgaris</i> ....	20	47	10	20	»	»	»	»	»	13
<i>Viola palustris</i> ....	13	55	48	66	60	20	»	»	»	24
<i>Agrostis tenuis</i> ....	30	90	93	70	48	»	»	»	»	15
<i>Oxycoccus quadrifidus</i> .....		70	»	27	23	»	»	»	»	9
<i>Hydrocotyle vulgaris</i>	»	90	90	53	40	70	»	»	»	9
<i>Agrostis canina</i> ....	»	100	100	73	63	100	»	»	»	12
<i>Festuca ovina</i> .....	»	100	47	35	20	20	50	»	»	12
<i>Anthoxanthum odoratum</i> .....	33	47	79	80	83	76	27	30	30	46
<i>Carex Goodenoughii</i>	30	70	100	55	90	65	10	70	60	18
<i>Holcus lanatus</i> ....	»	50	22	77	68	56	74	65	42	55
<i>Hieracium pilosella</i> ...	»	»	50	20	27	20	»	»	»	10
<i>Rumex acetosa</i> ....	23	30	43	32	48	60	44	50	»	50
<i>Carex panicea</i> .....	»	38	55	25	53	57	15	60	40	24
<i>Festuca rubra</i> .....	»	37	43	71	99	86	96	86	100	58
<i>Geum rivale</i> .....	»	»	60	70	78	89	70	53	25	37
<i>Plantago lanceolata</i> ...	»	»	45	33	63	80	53	50	30	30
<i>Salix repens</i> .....	»	20	30	43	10	20	»	»	»	10
<i>Luzula multiflora</i> ...	»	10	»	43	30	40	»	»	10	11
<i>Potentilla palustris</i> ...	»	15	23	41	68	30	20	»	»	21
<i>Galium palustre</i> ....	»	»	»	43	36	48	28	37	10	28
<i>Poa pratensis</i> .....	»	»	»	63	59	65	47	53	26	35
<i>Ranunculus acer</i> ....	»	»	13	53	62	58	51	90	63	45
<i>Veronica chamaedrys</i>	»	»	»	20	33	40	26	10	10	14
<i>Deschampsia caespitosa</i> .....	»	»	»	40	67	62	33	52	23	33
<i>Taraxacum</i> sp. ....	»	»	»	30	80	20	10	40	55	12
<i>Succisa pratensis</i> ...	»	»	10	10	10	50	25	34	»	12
<i>Cirsium palustre</i> ....	»	»	»	33	28	43	24	30	»	21
<i>Phragmites communis</i>	»	»	»	»	10	45	35	28	33	15
<i>Brunella vulgaris</i> ...	»	»	»	10	37	48	40	68	»	15
<i>Menta aquatica</i> ....	»	»	»	»	10	50	40	57	35	11
<i>Dactylis glomerata</i> ...	»	»	»	»	40	70	58	80	63	15
<i>Festuca elatior</i> ....	»	»	»	»	25	70	61	87	73	20
<i>Briza media</i> .....	»	»	»	»	25	50	45	48	100	11
<i>Filipendula ulmaria</i> ...	»	»	»	10	10	40	50	30	»	19
<i>Lathyrus pratensis</i> ...	»	»	»	»	40	37	55	73	50	11
<i>Cirsium oleraceum</i> ...	»	»	»	»	»	»	82	94	90	19
<i>Scirpus silvaticus</i> ...	»	»	»	»	»	»	50	100	80	8
<i>Trifolium pratense</i> ...	»	»	»	»	40	30	40	55	20	8
<i>Avena pubescens</i> ...	»	»	»	15	25	30	30	75	100	14
<i>Angelica silvestris</i> ...	»	»	»	»	»	35	33	48	30	14
<i>Carex glauca</i> .....	»	»	»	»	»	20	40	52	55	10
<i>Tussilago farfara</i> ...	»	»	»	»	»	10	10	55	80	9
<i>Agrostis alba</i> .....	»	»	»	»	»	»	30	65	60	7
<i>Carex hirta</i> .....	»	»	»	»	»	»	15	15	35	8
Average number of species.....	8.0	10.4	13.3	17.6	14.8	15.9	21.0	21.1	18.8	
Average density of species.....	4.4	5.8	5.9	8.4	8.0	8.3	9.4	10.6	8.7	

**Table 15.** Meadow plants. Number of occurrences in the pH<sup>r</sup> classes (per cent).

pH <sup>r</sup> classes.....	3.5-3.9	4.0-4.4	4.5-4.9	5.0-5.4	5.5-5.9	6.0-6.4	6.5-6.9	7.0-7.4	7.5-7.9	Number of occurrences
<i>Deschampsia flexuosa</i> .....	54	31	16	»	»	»	»	»	»	13
<i>Galium hircynicum</i> ..	41	18	20	5	6	»	»	»	»	18
<i>Molinia coerulea</i> ....	26	26	26	10	»	2	3	7	»	28
<i>Potentilla erecta</i> ....	20	20	20	20	6	4	3	8	»	39
<i>Calluna vulgaris</i> ....	31	24	31	14	»	»	»	»	»	13
<i>Viola palustris</i> .....	20	20	20	22	10	9	»	»	»	24
<i>Agrostis tenuis</i> .....	17	8	25	29	21	»	»	»	»	15
<i>Oxycoccus quadripetalus</i> .....	»	42	37	21	»	»	»	»	»	9
<i>Hydrocotyle vulgaris</i>	»	13	28	37	13	9	»	»	»	9
<i>Agrostis canina</i> .....	»	22	11	38	22	7	»	»	»	12
<i>Festuca ovina</i> .....	»	10	32	19	16	13	10	»	»	12
<i>Anthoxanthum odoratum</i> .....	8	8	20	20	17	14	7	3	3	46
<i>Carex Goodenoughii</i>	14	14	7	12	10	17	6	12	8	18
<i>Holcus lanatus</i> .....	»	5	14	12	15	12	15	12	15	55
<i>Hieracium pilosella</i> ..	»	»	27	36	20	17	»	»	»	10
<i>Rumex acetosa</i> .....	15	10	10	11	15	15	16	7	»	50
<i>Carex panicea</i> .....	»	20	10	26	10	10	9	9	6	24
<i>Festuca rubra</i> .....	»	7	7	15	15	15	14	14	13	58
<i>Geum rivale</i> .....	»	»	4	10	18	19	22	19	8	37
<i>Plantago lanceolata</i> ..	»	»	9	11	15	14	23	19	10	30
<i>Salix repens</i> .....	»	23	34	30	6	7	»	»	»	10
<i>Luzula multiflora</i> ...	»	12	»	42	24	8	»	»	17	11
<i>Potentilla palustris</i> ..	»	12	23	36	12	11	5	»	»	21
<i>Galium palustre</i> ....	»	»	»	13	20	29	18	13	6	28
<i>Poa pratensis</i> .....	»	»	»	10	17	19	20	13	22	35
<i>Ranunculus acer</i> ....	»	»	9	8	18	19	21	16	10	45
<i>Veronica chamaedrys</i>	»	»	»	9	19	12	41	9	11	14
<i>Deschampsia caespitosa</i> .....	»	»	»	4	21	24	14	18	19	33
<i>Taraxacum</i> sp.....	»	»	»	17	10	6	17	26	23	12
<i>Succisa pratensis</i> ...	»	»	10	17	5	6	17	44	»	12
<i>Cirsium palustre</i> ....	»	»	»	22	16	16	28	17	»	21
<i>Phragmites communis</i> .....	»	»	»	»	4	9	13	39	35	15
<i>Brunella vulgaris</i> ...	»	»	»	8	13	23	24	32	»	15
<i>Menta aquatica</i> .....	»	»	»	»	5	6	17	26	46	11
<i>Dactylis glomerata</i> ..	»	»	»	»	7	5	26	26	35	15
<i>Festuca elatior</i> .....	»	»	»	»	6	4	35	30	26	20
<i>Briza media</i> .....	»	»	»	»	11	15	20	40	14	11
<i>Filipendula ulmaria</i> ..	»	»	»	6	7	27	48	12	»	19
<i>Lathyrus pratensis</i> ..	»	»	»	»	12	22	21	31	14	12
<i>Cirsium oleraceum</i> ...	»	»	»	»	»	»	20	33	38	19
<i>Scirpus silvaticus</i> ...	»	»	»	»	»	»	58	12	30	8
<i>Trifolium pratense</i> ...	»	»	»	»	15	10	14	27	35	8
<i>Avena pubescens</i> ...	»	»	»	31	9	11	23	15	10	14
<i>Angelica silvestris</i> ..	»	»	»	»	»	10	27	27	36	14
<i>Carex glauca</i> .....	»	»	»	»	»	14	10	50	26	10
<i>Tussilago farfara</i> ....	»	»	»	»	»	7	11	41	41	9
<i>Agrostis alba</i> .....	»	»	»	»	»	»	12	24	64	7
<i>Carex hirta</i> .....	»	»	»	»	»	»	21	21	57	8

**Table 16.** Meadow plants. Number of occurrences with frequency 50 and upwards in the  $p_{H^+}$  classes (per cent).

$p_{H^+}$ classes.....	3.5-3.9	4.0-4.4	4.5-4.9	5.0-5.4	5.5-5.9	6.0-6.4	6.5-6.9	7.0-7.4	7.5-7.9	Num- ber of occu- rences
<i>Deschampsia flexuosa</i>	44	33	22	»	»	»	»	»	»	11
<i>Galium hircynicum</i>	54	36	11	»	»	»	»	»	»	10
<i>Molinia coerulea</i> ....	26	23	11	»	»	26	»	13	»	18
<i>Potentilla erecta</i> ....	22	22	19	14	11	11	»	»	»	28
<i>Viola palustris</i> .....	»	26	26	21	26	»	»	»	»	12
<i>Agrostis tenuis</i> .....	15	29	29	15	12	»	»	»	»	9
<i>Agrostis canina</i> .....	»	22	22	17	17	22	»	»	»	10
<i>Anthoxanthum odo- ratum</i> .....	9	9	18	19	22	23	»	»	»	30
<i>Holcus lanatus</i> .....	»	11	»	17	16	16	16	17	7	35
<i>Rumex acetosa</i> .....	»	»	10	16	21	27	12	14	»	18
<i>Festuca rubra</i> .....	»	»	5	12	17	15	17	17	17	50
<i>Geum rivale</i> .....	»	»	21	21	16	21	12	10	»	27
<i>Plantago lanceolata</i>	»	»	17	»	24	27	11	20	»	15
<i>Potentilla palustris</i> ..	»	»	13	16	54	18	»	»	»	8
<i>Galium palustre</i> ....	»	»	»	22	17	38	»	22	»	9
<i>Poa pratensis</i> .....	»	»	»	19	19	21	14	21	6	21
<i>Ranunculus acer</i> ....	»	»	»	9	15	16	17	26	18	27
<i>Deschampsia caesp- tosa</i> .....	»	»	»	»	28	32	12	28	»	16
<i>Dactylis glomerata</i> ..	»	»	»	»	13	27	20	20	20	11
<i>Festuca elatior</i> .....	»	»	»	»	»	32	18	26	24	13
<i>Filipendula ulmaria</i>	»	»	»	»	»	23	43	34	»	8
<i>Cirsium oleraceum</i> ..	»	»	»	»	»	»	30	35	35	18

tables contains the analytical results of a single locality, for which is stated the  $p_{H^+}$  value<sup>1)</sup> found in the aqueous extract of the soil sample, the depth of the level of the subsoil water below the surface of the meadow (the level of the subsoil water) at the time at which the investigation took place and the species<sup>2)</sup>

<sup>1)</sup> The  $p_{H^+}$  values given in the tables are those colorimetrically measured; besides all the extracts of the soil samples are also electrometrically measured, but the  $p_{H^+}$  values electrometrically found are not stated in the table.

<sup>2)</sup> At the formation statistical investigations only the vascular plants were noted. These are in the present work designated by the names found in Raunkjær's Danish excursion flora (1914).

found by the formation-statistical analysis in the selected trial areas, as also their frequency. At the bottom of the columns the number of species found in all the selected trial areas together with the density of the species (the average number of species on the trial area) are stated.

A single one of the localities examined, i. e. "Sortemose" at the western side of Farum Sea, will be more fully entered upon. The southern part of the Sortemose is a high moor partly covered with firs and birch-trees; the northern part on the other hand is a grass moor, on which *Cirsium oleraceum* is the dominant plant, and the soil of which is alkaline ( $p_{H^+}$  7.1—7.5). From this grass moor the hydrogen ion concentration of the peat increases over a distance of a few hundred metres evenly towards the high moor, the peaty soil of which is extremely acid ( $p_{H^+}$  3.6). Parallel to the alteration of the hydrogen ion concentration of the soil is an alteration of the composition of the vegetation. From the alkaline grass moor I wandered in the direction of the high moor, and with certain intervals an area of 2 m<sup>2</sup> was selected for investigation. The result hereof will be seen from the table 13, in which the analytical results are stated<sup>1)</sup>. It appears that the concentration of hydrogen ions of the peat increases towards the high moor and at the same time the features of the vegetation change. In the alkaline, the neutral and the slightly acid meadow ( $p_{H^+}$  7.5—6.8) *Cirsium oleraceum* is the physiognomically dominant species; besides *Briza media* and *Festuca elatior* form an essential part of the vegetation; nearer to the high moor, where the concentration of hydrogen ions of the peat is higher ( $p_{H^+}$  5.6—4.7) these species disappear and *Anthoxanthum odoratum*, *Agrostis canina*, *Viola palustris* and *Potentilla erecta* form an essential part of the vegetation. Near the high moor where the hydrogen ion concentration of the peat has a value of 4.7 in  $p_{H^+}$ , *Molinia coerulea* appears and becomes the dominant species. Where the hydrogen ion concentration of the peat expressed in  $p_{H^+}$  has a value of 4.2 or lower values, the high moor vegetation dominates in which *Eriophorum vaginatum*,

<sup>1)</sup> In table 13 the formations are contrary to the formations in the other tables arranged according to decreasing  $p_{H^+}$  values. This has been made because the alkaline grass moor in Sortemose in respect of development is the primary one, the high moor the secondary one, the latter having developed on the top of the grass moor. The table thus shows a succession.

*Oxycoccus quadripetalus*, *Calluna* and *Andromeda* figure prominently. It will be seen from table 13 that both the number of the species as also their density decrease with the increasing hydrogen ion concentration of the peat. Moreover it will be seen that the level of the subsoil water in all localities is the same; consequently the moisture conditions cannot be of any importance to the plant distribution. The analytical results stated in table 13 — the two high moor formations always excepted — besides also form part of the principal tables (tables 4—12), containing the result of all analysis of formations and habitats made on the meadow soil.

In order to obtain a summary of the distribution of the single species in relation to the hydrogen ion concentration of the soil on the basis of all the material examined by me, I have statistically treated the aggregate material of the tables 4—12. As mentioned above, the results of the analysis of the localities have been classed in 9 groups according to the  $p_{H^+}$  values found at the analysis of the meadow habitats, each group comprising a  $p_{H^+}$  range of 0.5. Based on the number of frequency in tables 4—12, I have for each single species computed its average frequency<sup>1)</sup> in each of the  $p_{H^+}$  classes in which it is found. The result will be seen from table 14, in which of course only such

<sup>1)</sup> When computing the average frequency of the species in the different  $p_{H^+}$  classes, regard has only been taken to the localities in which the species occur. The fact that a plant does not occur in a given locality, does not say anything of the ability of the species to thrive in this locality or not, as accidental circumstances may have involved that the plant has not got any opportunity whatever to grow here. If one desires to compute the average frequency of a given species by adding up the numbers of frequency for each single  $p_{H^+}$  class and divide with the total number of localities examined in the  $p_{H^+}$  class in question, an incorrect image of the distribution of the species would in many cases be obtained. A plant as *Hordeum europaeum* (the average frequency of which in the different  $p_{H^+}$  classes will be found in table 27 on the wood ground plants) thus is very frequent in the middle of Seeland, but is wanting in the north of Seeland. As the greater part of the wood habitats mentioned in the following and examined by me are taken from the north of Seeland, it will be seen that if regard is paid to the total number of the localities examined when computing the average frequency, figures would be come at which entirely would depend on the number of localities examined in the north of Seeland, where the species is not found. If furthermore a great number of localities is examined in the north of Seeland, and if these localities preferably fall on single  $p_{H^+}$  classes it will easily be understood that the average thus come at will be quite other than that first reached and by no means proportional to those first come at.



species are mentioned which are found in so many of the localities that a statistical treatment of the numbers of frequency is possible. Each figure in table 14 thus is an average number of all the numbers of frequency for a single species in a single  $p_{H^+}$  class. Besides it is a matter of course that the table also states in which  $p_{H^+}$  classes the species mentioned in the table are found. By means of the figures a graph may be drafted for each single species; the apex of the graph is in the  $p_{H^+}$  class in which the species has its greatest frequency. An even graph is not always obtainable for all species. In some cases the graph may have an irregular course, which i. a. is due to the fact that not all the figures are averages of a sufficient number of frequency. If this was the case (several Hundred of localities should then have been examined) regular graphs would certainly have appeared. However, it must be noted that several of the irregular graphs, e. g. the graphs for *Festuca ovina* and *Taraxacum sp.*, have two apices. This may perhaps follow from the fact that it is a question of two polymorpheous species, the two apices severally may form the apex of a single graph representing a single race of the species. In the table is stated for each species in how many out of the 76 localities examined it occurs. Moreover by aid of the tables 4—12 it will easily be seen on how many figures each of the averages in table 14 is based.

As will be seen from table 14, the single species are arranged in such a way that the species having the greatest frequency on the most acid soils are set first, while the species most frequently found on alkaline soil are set at the bottom. The species mentioned first in the table are the "lime-avoiding" plants of the elder authors (e. g. *Deschampsia flexuosa* and *Calluna vulgaris*), those at the bottom their "lime-loving" plants (e. g. *Tussilago farfarus*).

In table 14 will moreover be found for each single  $p_{H^+}$  class the average of the number of species found in the localities examined as also the average of their density. It will be seen that both the number of the species as their density on an average is greater on habitats with neutral-slightly alkaline soil ( $p_{H^+}$  7.0—7.4), and that both the number of the species and their density decrease with increasing hydrogen ion concentration of the soil; on the very alkaline soils ( $p_{H^+}$  7.5—8.0) both the number of the species as their density are on an average lower

than on the neutral-slightly alkaline soils. By means of the averages for both the number of the species as the density hereof a graph may be drawn, the apex of which is near the neutral point.

Furthermore I have examined as regards the single species how the number per cent. of occurrences — without regard to the frequency — is distributed on the 9  $p_{H^+}$  classes. The result will be seen from table 15. By aid of the figures in this table a graph may also be drawn for each species, the apex of which is in the  $p_{H^+}$  class in which the species is most often found. Moreover, when comparing this table with table 14<sup>1)</sup>, it will be seen that the single species as a rule occur most frequently in the same  $p_{H^+}$  classes in which they have their greatest average frequency.

In table 16 will finally be found for the single species a summary of how the per cent. number of occurrences with 50 or more as number of frequency is distributed on the 9  $p_{H^+}$  classes<sup>2)</sup>.

It will appear from table 14, 15 and 16 that the single species only are found on soil the hydrogen ion concentration of which lies within certain limits, i. e. within a certain range of  $p_{H^+}$ , and that this range differs as regards the different species. Some species are seen to appear within a more limited range than others. The species the  $p_{H^+}$  of which is narrowly limited may, therefore, when very frequently found serve as a kind of indicator of the hydrogen ion concentration of the soil. Thus *Cirsium oleaceum*, *Angelica silvestris*, *Agrostis alba* and *Tussilago farfarus* will indicate that the hydrogen ion concentration of the soil expressed in  $p_{H^+}$  lies between 6.5 and 8.0, *Deschampsia caespitosa*, especially where large tussocks are formed, indicates the hydrogen ion concentration to be 5.5 and 6.5 and finally *Molinia coerulea* and *Deschampsia flexuosa* indicate that the hydrogen ion concentration is between 3.8 and 4.5. On the other hand no single species is found which might especially serve as indicator of the  $p_{H^+}$  range 4.5—5.5.

<sup>1)</sup> The species in table 15 are arranged in the same succession as in table 14.

<sup>2)</sup> When computing the figures in table 15 and 16 (as also in the following table 28) regard has been paid to the fact that equal numbers of localities have not been examined in each  $p_{H^+}$  class, which has not been made in the Danish edition. Thus the figures of these three tables do not agree with those in the Danish edition.

**Determination of the hydrogen ion concentration of the meadow soil through the vegetation.** A more minute determination of the hydrogen ion concentration of the meadow soil through the vegetation can, however, be made when using for this purpose not a single species, but a greater number of species found, and when regard is taken to the frequency with which they are found. In this case it will be necessary on the spot to make a formation-statistical analysis. For such an analysis, however, it is sufficient to select 5 trial areas. At these determinations I have made use of a method which will appear from the following 3 instances.

The first instance is an analysis of a formation taken from a meadow south of Jægersborg deer-park. 5 trial areas were altogether selected. The species found and their frequency were then noted in the two first columns of a table as the following one; in this table will besides be found 9 columns corresponding to the above mentioned 9  $p_{H^+}$  classes. As regards *Festuca rubra*, the first stated species, the frequency of which is 100, table 14 was then examined to see in which  $p_{H^+}$  classes this species has an average frequency<sup>1)</sup> near 100, and as this was found to be the case for the last 5  $p_{H^+}$  classes, a level line was drawn through the last 5 columns off the species. Then I continued with the other species in the same manner, provided that the species was found in table 14, and if so only provided a fairly regular "graph" was found, which e. g. is not the case as regards *Taraxacum*. Then the number of the lines for each  $p_{H^+}$  class was added up, and the hydrogen ion concentration of the meadow soil expressed in  $p_{H^+}$  should then be in the  $p_{H^+}$  class for which the highest number of lines was found, in this case in the  $p_{H^+}$  class 7.5—7.9. A soil sample taken on the locality in question also gave a colorimetrically measured  $p_{H^+}$  value of 7.6.

The second instance of such a determination made on a meadow locality in Strogaards Vang (Grib Forest) is shown in the following table (instance 2). Also in this case only 5 trial areas were selected at the formation-statistical examination. As

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<sup>1)</sup> It is a matter of course that in many cases it depends on a judgement whether an average number of frequency entered in table 14 for a given species is to be considered as being near the figure found for the species in the formation examined or not. Fixed rules may of course be given here, but for the present I have not deemed it necessary.

## Instance 1. Meadow locality south of Jegersborg deer-park.

Species	No. of frequency	pH classes								
		3.5—3.9	4.0—4.4	4.5—4.9	5.0—5.4	5.5—5.9	6.0—6.4	6.5—6.9	7.0—7.4	7.5—7.9
<i>Festuca rubra</i> .....	100	.	.	.	.	.	—	—	—	—
<i>Festuca elatior</i> .....	100	.	.	.	.	.	—	—	—	—
<i>Briza media</i> .....	100	.	.	.	.	.	—	—	—	—
<i>Avena pubescens</i> .....	100	.	.	.	.	.	.	.	.	.
<i>Ranunculus acer</i> .....	100	.	.	.	.	.	.	.	.	.
<i>Linum catharticum</i> .....	100	.	.	.	.	.	.	.	.	.
<i>Cirsium oleraceum</i> .....	100	.	.	.	.	.	.	.	.	.
<i>Taraxacum</i> sp. ....	100	.	.	.	.	.	.	.	.	.
<i>Scirpus sylvaticus</i> .....	80	.	.	.	.	.	.	.	.	.
<i>Carex glauca</i> .....	80	.	.	.	.	.	.	.	.	.
<i>Phragmites communis</i> .....	60	.	.	.	.	.	.	.	.	.
<i>Phleum pratense</i> .....	60	.	.	.	.	.	.	.	.	.
<i>Potentilla anserina</i> .....	40	.	.	.	.	.	.	.	.	.
<i>Geran rivale</i> .....	40	.	.	.	.	.	.	.	.	.
<i>Plantago lanceolata</i> .....	40	.	.	.	.	.	.	.	.	.
<i>Avena pratensis</i> .....	20	.	.	.	.	.	.	.	.	.
<i>Alenta aquatica</i> .....	20	.	.	.	.	.	.	.	.	.
<i>Hypericum acutum</i> .....	20	.	.	.	.	.	.	.	.	.
		2	1	2	3	4	9	10		

**Instance 2.** Meadow locality in Strøgaards Vang (Grib Forest).

Species	No. of frequency	pH classes							
		3.5—3.9	4.0—4.4	4.5—4.9	5.0—5.4	5.5—5.9	6.0—6.4	6.5—6.9	7.0—7.4 7.5—7.9
<i>Festuca rubra</i> .....	100	.	.	.	.	—	—	—	—
<i>Holcus lanatus</i> .....	100	.	.	.	—	—	—	—	—
<i>Anthoxanthum odoratum</i> .....	100	.	.	—	—	—	—	—	—
<i>Geum rivale</i> .....	100	.	.	.	—	—	—	—	—
<i>Plantago lanceolata</i> .....	100	.	.	.	.	—	—	—	—
<i>Rumex acetosa</i> .....	60	.	.	.	.	—	—	—	—
<i>Galium palustre</i> .....	40	.	.	.	—	—	—	—	—
<i>Poa pratensis</i> .....	20	.	.	.	.	—	—	—	—
<i>Lathyrus pratensis</i> .....	20	.	.	.	.	—	—	—	—
<i>Taraxacum</i> sp.....	20	.	.	.	.	—	—	—	—
		1	1	1	4	8	8	5	4 2

## Instance 3. Locality No. 14 (see table 5).

Species	No. of frequency	pH classes								
		3.5—3.9	4.0—4.4	4.5—4.9	5.0—5.4	5.5—5.9	6.0—6.4	6.5—6.9	7.0—7.4	7.5—7.9
<i>Molinia coerulea</i> .....	100	—	—							
<i>Potentilla erecta</i> .....	100	.	—							
<i>Deschampsia flexuosa</i> .....	40	.	.	—						
<i>Anthoxanthum odoratum</i> .....	40	—	—	.	.	.	.	.	—	—
<i>Oxycochus quadrifidatus</i> .....	40	—	—		.	.	.	.		
<i>Festuca rubra</i> .....	40	.	—	—						
<i>Kumex acetosa</i> .....	30	.	.	—	—					
<i>Holcus lanatus</i> .....	20	.	.	—						
<i>Carex panicea</i> .....	10		—							
<i>Potentilla palustris</i> .....	10	.	.	—						
<i>Peucedanum palustre</i> .....	10		—							
<i>Achillea ptarmica</i> .....	10									
		2	6	3	1			1	1	1

will appear from the table the result was that the hydrogen ion concentration of the meadow soil expressed in  $p_{H^+}$  probably was near 6, the  $p_{H^+}$  class 5.5—5.9 being as probable as that 6.0—6.4. A soil sample taken on the spot had the  $p_{H^+}$  value 5.8.

Finally the third instance (page 62) is stated. For this determination one of the localities included in the preceding tables, i. e. locality no. 14 (see table 5) was used in the soil of which the hydrogen ion concentration expressed in  $p_{H^+}$  was found to be 4.4.

By means of the said procedure I have "determined" the soil on a number of meadow localities in the north of Seeland, these localities as those in instance 1 and 2 are not included in the material, on which table 14 is based, and controlled the result by aid of the soil samples selected. Furthermore, I have tried the procedure on the majority of the formations mentioned in tables 4—12, the hydrogen ion concentration of their habitats being known beforehand. On the basis of these examinations it can be said that through the said procedure the hydrogen ion concentration in most cases can be determined in the "right"  $p_{H^+}$  class. There are, however, cases where the right  $p_{H^+}$  class is not found, i. e. the  $p_{H^+}$  class in which lies the  $p_{H^+}$  value of the soil sample measured, but a  $p_{H^+}$  class immediately to the right or to the left of the correct one; this seems most often to be the case when the hydrogen ion concentration of the soil expressed in  $p_{H^+}$  has a value from 5.0 to 5.5. I cannot tell whether the numerical value stated in table 14 may be used at the determination of the hydrogen ion concentration in the above mentioned manner, e. g. also in West Jutland, that differs rather much from the north of Seeland.

As mentioned before, the level of the subsoil water has been measured in the places examined. These measurements will be seen from tables 4—12. As the level of the subsoil water varied in the different localities from 30—100 cm, such different level has of course somewhat influenced the composition of the vegetation. Some species, as e. g. *Carex Goodenoughii* and *Hydrocotyle vulgaris* as a rule only are found where the subsoil water is found in a small depth (about 30 cm). A statistical treatment of the material examined clearly shows that the different levels of the subsoil water influence the average density of the species. When classifying the different localities in 8 classes according

to the level of the subsoil water and when calculating the average density of the species of each class, the following figures will be reached<sup>1)</sup>:

Classes of the level of the subsoil water (cm)	30-39	40-49	50-59	60-69	70-79	80-89	90-99	100-109
Average density of the species .....	9.3	8.3	7.5	7.1	6.7	4.8	»	7.5

It will be seen that the average density of the species decreases with the increasing depth of the level of the subsoil water; only the last class forms an exception, but in so great depth (100 cm) the subsoil water has presumably no influence or only a small one on the density of the species; it must therefore be supposed that the density in the latter case is determined by other factors.

In case the average level of the subsoil water is calculated for the localities examined in the above mentioned 9  $p_{H^+}$  classes, it will appear to vary rather considerably in the different  $p_{H^+}$  classes, and it is obviously that this fact has influenced the figures given in table 14 for the different  $p_{H^+}$  classes as regards the average density of the species. These figures are stated here together with the average level of the subsoil water in the different  $p_{H^+}$  classes.

$p_{H^+}$ classes .....	3.5-3.9	4.0-4.4	4.5-4.9	5.0-5.4	5.5-5.9	6.0-6.4	6.5-6.9	7.0-7.4	7.5-7.9
Average level of the subsoil water	53	40	54	48	66	69	69	50	52
Average density of the species .....	4.4	5.8	5.9	8.4	8.0	8.3	9.4	10.6	8.7

Especially in the second and fourth  $p_{H^+}$  class the average level of the subsoil water will be seen to be considerably lower than in the other classes, and the figures of the average density of the species will consequently be too high in the said  $p_{H^+}$  class. By means of the above summary of the average density of the species in the 8 classes of the level of the subsoil water, the figures of the density in the  $p_{H^+}$  classes can, however, be corrected, as it will be seen here how great the influence of a certain alteration of the level of the subsoil water has on the density of the species. If the figures of the density of the species

<sup>1)</sup> None of the localities examined occur in the last but one class of the level of the subsoil water.



in the different  $p_{H^+}$  classes are corrected in such a way that in all the  $p_{H^+}$  classes an average level of 60–69 cm is calculated, the following figures of the density of the species in the 9  $p_{H^+}$  classes will be reached.

$p_{H^+}$ classes . . . . .	3.5-3.9	4.0-4.4	4.5-4.9	5.0-5.4	5.5-5.9	6.0-6.4	6.5-6.9	7.0-7.4	7.5-7.9
Average density of species (corr.)..	4.0	4.6	5.5	7.2	8.0	8.3	9.4	10.2	8.3

These figures will be seen to represent a considerably finer graph than those stated above.

In 15 of the localities examined soil samples were taken out to examine the course of the transformation processes of the nitrogen. The result will be seen from the following table. It has not been possible to deduce any connection between the activity of the nitrification and the composition of the vegetation. It will also be seen from the table that there does not exist a closer connection between the hydrogen ion concentration of the

**Table 17.** The transformation processes of the nitrogen in meadow soils.

Locality	$p_{H^+}$	per cent $H_2O$	In 1 kilo dry soil was found mg $NO_2$		In 1 kilo dry soil was found mg $NH_4$	
			at once	after one month	at once	after one month
No. 78 table 13. . . . .	3.6	87	0	0	15	14
- 77 - 13. . . . .	3.8	82	0	0	12	14
- 5 - 4. . . . .	3.8	80	8	37	20	42
- 6 - 4. . . . .	3.8	85	29	62	6	5
- 12 - 5. . . . .	4.4	80	100	215	40	65
- 14 - 5. . . . .	4.4	55	27	40	8	10
- 16 - 6. . . . .	4.6	48	98	184	5	7
- 25 - 7. . . . .	5.1	52	184	216	7	5
- 32 - 8. . . . .	5.5	56	70	185	4	8
- 43 - 8. . . . .	5.9	30	40	95	4	4
- 45 - 9. . . . .	6.0	41	32	128	3	7
- 48 - 9. . . . .	6.2	52	17	216	not determined	
- 61 - 10. . . . .	6.8	41	85	211	5	3
- 74 - 12. . . . .	7.5	56	22	45	4	3
- 76 - 12. . . . .	7.7	42	30	130	6	5

soil and the intensity of the nitrification. Only in the two first mentioned localities from high moor no nitrification whatever takes place, but the hydrogen ion concentration of the peat in one of these localities is not higher than in the peat in the two following *Molinia* meadows (loc. 5 and 6), in the soil of which the nitrification takes place. Thus nitrification apparently takes place in all meadow soils, even in the most acid ones, while the high moor peat is not nitrifying. Besides it appears from the table that the greatest quantities of ammonia are found in the most acid soils.

In none of the meadow soils examined the mineral plant nutrients have been determined.

### **The hydrogen ion concentration of the wood soil and the distribution of the wood ground plants.**

As mentioned in the section on the meadows it is expedient, when desiring to examine the distribution of the plants in relation to a single factor — in this case the hydrogen ion concentration of the soil — that the habitats examined differ as little as possible as regards all other factors. In the wood ground the conditions of light, as will be known, play a great part as a plant distributing factor. Therefore it would answer the purpose, if the conditions of light were fairly uniform in the localities selected for examination. However, it soon appeared too difficult only to examine localities within certain conditions of light, i. a. because in this case measurements of the light had to be made on a very great number of localities, among which such were to be selected for examination in which the conditions of light were fairly the same. Although however the conditions of light in a number of localities are found to be homogeneous, it is — as mentioned above — by no means given that the vegetation in all the localities are in equilibrium with the conditions of light prevailing at the moment, because these conditions on account of cuttings frequently being made rarely are homogeneous in the same locality for a longer number of years. At the examination, therefore, localities have been included in which the conditions of light were rather different. On some of the localities measurements of the light have been made with Wynnés'

Table 18. Analysis of wood ground habitats and their vegetation.

pH 3.5—3.9.

Locality no. 84 is situated in Hareskov, no. 92 in Rude Forest, the others in Grib Forest. (The quantity of  $\text{NO}_3$ ,  $\text{NH}_4$ , K,  $\text{PO}_4$  and Ca in the soil is expressed in mg per one kilo dry soil).

Part of the soil sample	3-5	3-5	3-5	3-6	3-7	3-8	3-8	3-8	3-8	3-9	3-9	3-9	3-9
Locality no.	79	80	81	82	83	84	85	86	87	88	89	90	91
<i>Luzula pilosa</i> .....	100	80	100	100	100	100	100	100	100	100	50	100	70
<i>Deschampsia flexuosa</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Convallaria majalis</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Maianthemum bifolium</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Vaccinium myrtillus</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Pteridium aquilinum</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Carex pilulifera</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Molinia coerulea</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Milium effusum</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Stellaria holostea</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Anemone nemorosa</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Oxalis acetosella</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Vaccinium vitis idaea</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Melampyrum vulgatum</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Galium hircynicum</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
<i>Hieracium umbellatum</i> .....	100	80	100	100	100	100	100	100	100	100	100	100	100
Number of species .....	2	4	3	2	3	2	3	3	2	4	3	4	4
Density of species .....	2.0	2.0	2.1	1.1	1.3	2.0	2.1	1.6	1.2	1.4	1.3	1.8	0.8
Light percentage .....	6	38	38	6	38	38	38	38	38	38	38	38	38
per cent $\text{H}_2\text{O}$ .....	40	38	38	45	38	38	38	38	38	38	38	38	38
$\text{NO}_3$ at once .....	0	0	0	0	0	0	0	0	0	0	0	0	0
— after one month .....	0	0	0	0	0	0	0	0	0	0	0	0	0
$\text{NH}_4$ at once .....	10	4	4	10	4	4	4	4	4	4	4	4	4
— after one month .....	51	107	107	60	4	4	4	4	4	4	4	4	4
K .....	8	3	3	25	3	3	3	3	3	3	3	3	3
$\text{PO}_4$ .....	8	3	3	25	3	3	3	3	3	3	3	3	3
Ca .....	8	3	3	27	3	3	3	3	3	3	3	3	3

In the soil sample



TABLE 20. Analysis of wood ground habitats and their vegetation.

pH 4.5—4.9.

Locality no. 115 is situated in Jægersborg deer park, nos. 116, 120, 121, 122, 127, 131, 132, 135 and 137 in Hareskoven, nos. 117, 123, 124, 125, 126, 130 and 134 in Grå Forest, nos. 118 and 136 in Store Bøgeskov; nos. 119, 128 and 133 in Ernehunden and no. 129 in Jonstrup Vang. (The quantity of  $\text{NO}_3$ ,  $\text{NH}_4$ , K,  $\text{PO}_4$  and Ca in the soil is expressed in mg per one kilo dry soil).

pH of the soil sample	4.5	4.5	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.7	4.7	4.7	4.7	4.7	4.8	4.8	4.8	4.8	4.9	4.9		
Locality no.	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137
Melica uniflora.....			100				100			100		100	100			100				100		100	100
Milium effusum.....						30	80					80			10							80	20
Majanthemum bifolium.....												40				70							
Stellaria holostea.....												40				30	100	100	100	30	100	100	70
Anemone nemorosa.....	100	100	60	100	100	100	60	100		100		100	100	100	100	30	100	100	100	30	100	100	
Viola silvestris.....			50				10					10					20					30	
Mercurialis perennis.....										50		10											
Oxalis acetosella.....	100		30	100	100	100	30	100			100	80			10	90	100	90	30	50	100	80	
Geranium Robertianum.....																		10			100		
Lamium galeobdolon.....																							
Asperula odorata.....	10		10				50			100		30	100	50		50				10	100	90	40
Carex silvatica.....																						10	
Deschampsia caespitosa.....												10											
Gagea lutea.....						30													20				
Ranunculus auricomus.....													10										
Anemone hepatica.....						10																	
— ranunculoides.....						10																	
Corydalis cava.....															10				10				
— intermedia.....																							
Veronica montana.....																							
Stachys silvaticus.....																							
Lapsana communis.....																10			10				
Number of species.....	3	1	5	4	4	3	7	1	2	2	4	7	5	1	4	7	2	3	6	4	3	10	5
Density of species.....	2.1	1.0	2.5	3.1	1.5	2.2	3.5	1.0	2.0	1.5	2.4	3.8	3.4	1.0	1.3	3.8	1.2	2.1	2.4	1.7	2.5	6.6	3.1
Light percentage.....		4	3				10								4								
per cent H <sub>2</sub> O.....		21	26				26								22								
NO <sub>3</sub> at once.....		25	13				10								15								
— after one month.....		382	385				412								188								
NH <sub>4</sub> at once.....		6	11				12								3								
— after one month.....		16	57				16								2								
K.....							24																
PO <sub>4</sub> .....							2																
Ca.....							42																

In the soil sample



-5.4.

.1	.1	.1	.2	.2	.2	.2	.2	.2	.3	.3	.3	.3	.3	.3	.4	.4	.4	.4	.4	.4
49	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	
00	>	100	100	30	20	>	>	>	100	>	100	100			100	100	100			
>	>	>	>	100	>	20	10	>	>	>			>			"	60			
>	>	>	>		80	>	>	>	>	>	>	>	>	>	>	>				
>	>	>	>		>	>	>	>	>	>	>	10	1	>	>	>				
>	>	>	>	>	>	>	>	>	>	40	>	>	>	>	>	>				
50	100	>	>		100	100	20	80	60	100	50	20	>	>	70	100	40	100	100	
>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>				
>	>	>	10	>	>	>	>	>	60	>	>	20								
>	>	20	>	60	>	>	>	100	>				>	>	>	>	>	>	>	
100	20	>	60	100	100	100	100	100	20	100	100	30	30	100	30	100	>	30	100	
>	>	>	>	>	>	>	>	>	>	>	>	>	100				>	>	>	
60	>	90	20	>	20	>	>	100	>	100	50	50	20		70	50	>	100		

3	2	3	5	3	9	3	5	3	4	7	4	6	6	4	4	5	1	2	3
2.1	2.0	2.1	1.6	1.9	4.4	2.2	1.7	2.8	2.8	4.2	3.0	2.2	2.1	2.8	3.0	3.5	1.0	1.3	3.0
»	»	»	»	»	»	»	»	2	»	»	»	10	»	»	»	»	»	5	»
»	»	»	»	»	»	»	»	23	»	»	»	19	»	»	»	»	»	32	»
»	»	»	»	»	»	»	»	0	»	»	»	9	»	»	»	»	»	5	»
»	»	»	»	»	»	»	»	191	»	»	»	240	»	»	»	»	»	242	»
»	»	»	»	»	»	»	»	6	»	»	»	5	»	»	»	»	»	20	»
»	»	»	»	»	»	»	»	7	»	»	»	30	»	»	»	»	»	69	»
»	»	»	»	»	»	»	»	»	»	»	»	17	»	»	»	»	»	»	»
»	»	»	»	»	»	»	»	»	»	»	»	2	»	»	»	»	»	»	»
»	»	»	»	»	»	»	»	»	»	»	»	40	»	»	»	»	»	»	»









[illegible]



[illegible]

Table 25. Analysis of wood ground habitats and their vegetation.

pH: 7.0—7.4.

Locality nos. 234, 235, 241, 242 and 244 are situated in Store Bogeskov, nos. 236 and 247 in Norteskov near Farum, nos. 237, 238, 239 and 240 in Allindille Fredskov, no. 243 in Boserup wood, no. 245 in Ermelunden and no. 246 in Bogues Storskov. (The quantity of  $\text{NO}_2$ ,  $\text{NH}_4$ , K,  $\text{PO}_4$  and Ca in the soil is expressed in mg per one kilo dry soil).

pH of the soil sample.....	7.0	7.0	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.4	7.4	7.4	7.4
Locality no.....	234	235	236	237	238	239	240	241	242	243	244	245	246	247
<i>Melica uniflora</i> .....	»	10	»	20	»	100	»	»	»	10	60	»	»	»
<i>Brachypodium sylvaticum</i> .....	»	»	100	»	»	»	»	»	»	»	»	»	»	100
<i>Hordeum europaeum</i> .....	100	100	»	70	100	»	100	100	100	»	100	»	100	»
<i>Allium ursinum</i> .....	»	»	»	»	»	»	»	»	»	»	»	»	»	»
<i>Ficaria verna</i> .....	»	»	»	»	»	»	»	»	»	100	»	»	»	40
<i>Anemone hepatica</i> .....	»	»	»	80	80	80	80	40	»	20	»	»	»	»
— <i>nemorosa</i> .....	»	100	»	70	»	»	»	100	100	100	100	»	»	»
<i>Viola silvestris</i> .....	10	40	30	40	10	80	50	60	10	»	10	»	»	»
<i>Mercurialis perennis</i> .....	»	100	»	100	100	100	20	»	100	100	»	»	20	»
<i>Oxalis acetosella</i> .....	100	30	10	»	10	»	90	90	40	»	100	»	»	20
<i>Ceanothus urbanum</i> .....	»	»	20	10	»	»	»	»	»	50	»	50	»	40
<i>Vicia sepium</i> .....	»	»	30	»	»	»	50	»	»	»	20	»	»	40
<i>Hedera helix</i> .....	»	»	»	80	40	50	»	»	»	»	»	»	»	»
<i>Aegopodium podagraria</i> .....	»	»	»	»	»	»	»	»	»	80	»	100	»	»
<i>Adoxa moschatellina</i> .....	»	»	»	»	»	»	»	»	»	100	»	»	»	»
<i>Asperula odorata</i> .....	»	10	»	20	40	40	90	100	10	»	»	10	»	»
<i>Festuca gigantea</i> .....	»	»	»	»	20	40	»	»	»	»	»	»	»	»









Table 27. The average frequency of the wood plants in the Ppr classes.

pr. classes	3.5—3.9	4.0—4.4	4.5—4.9	5.0—5.4	5.5—5.9	6.0—6.4	6.5—6.9	7.0—7.4	7.5—7.9	Number of occurrences
<i>Vaccinium myrtillus</i> .....	100	86	"	"	"	"	"	"	"	8
<i>Deschampsia flexuosa</i> .....	76	86	"	"	"	"	"	"	"	22
<i>Carex pilulifera</i> .....	18	14	"	"	"	"	"	"	"	9
<i>Luzula pilosa</i> .....	50	34	"	"	10	"	"	"	"	11
<i>Convallaria majalis</i> ...	100	68	"	"	"	"	"	"	"	6
<i>Majanthemum bifolium</i> .....	60	93	55	30	"	"	"	"	"	13
<i>Trichialis europaea</i> .....	"	30	"	"	"	"	"	"	"	4
<i>Oxalis acetosella</i> .....	20	62	74	76	78	61	65	54	43	87
<i>Anemone nemorosa</i> .....	10	61	88	76	77	91	94	95	65	113
<i>Launium galeoides</i> .....	"	100	100	100	100	100	43	40	80	13
<i>Melica uniflora</i> .....	"	55	100	88	76	42	43	40	20	51
<i>Milium effusum</i> .....	20	35	50	47	50	10	10	"	"	20
<i>Asperula odorata</i> .....	"	25	53	61	85	10	34	40	36	29
<i>Hordeum europaeum</i> .....	"	"	"	80	75	85	100	93	70	23
<i>Mercurialis perennis</i> .....	"	"	30	70	87	81	80	80	87	53
<i>Viola silvestris</i> .....	"	20	24	22	27	18	34	100	20	45
<i>Allium ursinum</i> .....	"	"	"	"	100	85	100	100	100	14
<i>Agropodium podagraria</i> .....	"	"	"	"	55	100	100	90	81	7
<i>Brachypodium silvaticum</i> .....	"	"	"	"	"	100	100	100	30	15
<i>Ficaria verna</i> .....	"	"	"	"	"	"	65	70	23	6
<i>Clem. urbanum</i> .....	"	"	"	"	"	20	35	34	59	16
<i>Anemone hepatica</i> .....	"	"	15	15	15	10	25	59	63	23
<i>Sanicula europaea</i> .....	"	"	"	"	"	20	23	30	43	16
Average number of species.....	2.9	4.6	4.0	4.4	3.8	3.7	4.5	8.4	5.7	
— density of species.....	1.6	2.4	2.4	2.6	2.3	1.9	2.5	3.9	2.7	

**Table 28.** Wood ground plants. Number of occurrences with frequency of 50 and more than 50 in the pH-classes (per cent).

pH-classes	3.5-3.9	4.0-4.4	4.5-4.9	5.0-5.4	5.5-5.9	6.0-6.4	6.5-6.9	7.0-7.4	7.5-7.9	Number of occurrences
<i>Vaccinium myrtillus</i> .....	100	»	»	»	»	»	»	»	»	8
<i>Deschampsia flexuosa</i> .....	45	55	»	»	»	»	»	»	»	18
<i>Convallaria majalis</i> .....	57	43	»	»	»	»	»	»	»	4
<i>Majanthemum bifolium</i> .....	20	40	20	20	»	»	»	»	»	10
<i>Oxalis acetosella</i> .....	»	15	15	14	15	13	13	9	7	58
<i>Anemone nemorosa</i> .....	»	10	13	12	11	13	15	15	11	92
<i>Lanium galeobdolon</i> .....	»	20	20	20	20	20	»	»	»	12
<i>Melica uniflora</i> .....	»	12	23	20	17	8	12	9	»	33
<i>Milium effusum</i> .....	»	11	31	27	31	»	»	»	»	9
<i>Asperula odorata</i> .....	»	7	16	20	25	6	11	7	8	36
<i>Hordeum europaeum</i> .....	»	»	»	19	19	14	19	19	12	20
<i>Mercurialis perennis</i> .....	»	»	9	14	16	15	14	14	18	44
<i>Allium ursinum</i> .....	»	»	»	»	21	17	21	21	21	13
<i>Aegopodium podagraria</i> .....	»	»	»	»	14	29	29	29	»	6
<i>Brachypodium sylvaticum</i> .....	»	»	»	»	»	26	26	26	23	14
<i>Anemone hepatica</i> .....	»	»	»	»	»	»	»	50	50	8

actinometre, and the values of light here oscillated between 2 and 11 per cent. of the free day light. In the localities, where the light was not measured the conditions of light have presumably been within the same limits.

All the localities examined are situated in forests of beech-trees. Furthermore the soil in all the localities is a mineral soil. Localities have been avoided, where the subsoil water is found in inconsiderable depth and where the soil consequently is rather moist. I have also avoided localities with very dry soil. The formations examined, therefore, all are found in habitats with fairly uniform conditions of moisture, which also will appear from the values of the water contents<sup>1)</sup> of the soil, given in the following tables for some of the localities examined.

The investigations were made during the years 1917—1920. The result of the analysis will appear from the tables 18—26, in which the formations examined are arranged according to the hydrogen ion concentration<sup>2)</sup> of the soil on the habitats and on the whole in the same manner as in the tables on the meadow localities (tables 4—12). As will be seen from the tables the majority of the localities are situated in the north of Seeland, but also localities from the middle of Seeland, from Fünen, Lolland-Falster and Jutland are included in the investigations. For each locality the species found at the formation-statistical ana-

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<sup>1)</sup> An exact basis for a comparison of the conditions of moisture in the different habitats will not be obtained by these figures, for although the soil samples — as mentioned before — were neither selected in specially dry periods nor in specially moist ones, they were not selected at the same time on all the localities. Even if this had been the case, rainfalls in some of the localities a short time before the samples were taken out might have caused that the soils as regards the conditions of moisture could not be compared. Finally, it must be borne in mind that only a fraction of the water found in the soil is accessible for the plants, and this fraction differs in the different soils, as it depends on the water capacity of the soil, i. e. the ability of the soil to keep the water. The capacity of clay soil thus is considerably greater than that of a sandy soil. Clay soil and sandy soil both containing e. g. 14 per cent. water thus are not equally moist for the plants, as a far greater part of the water may be absorbed by the plants on the sandy soil than on the clay soil. A suitable method for the determination of the water capacity of the soil does not exist; on the whole there is no method by which the conditions of moisture of two habitats can be compared with each other.

<sup>2)</sup> The hydrogen ion concentration was measured electrometrically for all the soil samples, half of the samples also colorimetrically. The  $p_{H^+}$  values stated in the tables are those electrometrically measured.

lysis are stated in the tables, as also their number of frequency together with the number and the density of the species; besides is stated for some of the localities examined the light value measured and the result of the investigation of the transformation processes of the nitrogen in the soil. For a few localities a determination has been made of the quantities of potassium, phosphate and calcium found in the soil and soluble in distilled water saturated with carbonic acid. The result of these investigations will also be seen from the tables.

On the basis of all the material in the tables 18—26 the average frequency of the single wood ground species in each of the before mentioned 9  $p_{H^+}$  classes has been made out, and the result will be seen from table 27, which has been elaborated in the same way as table 14. It appears from this table that *Vaccinium myrtillus*, *Deschampsia flexuosa*, *Carex pilulifera*, *Luzula pilosa*, *Convallaria majalis*, *Majanthemum bifolium* and *Triantalis europaea* are the species found most frequently in the most acid wood soils. These species are besides nearly the same as those described by Müller as characteristic for the raw humus soil. On the middle acid wood soil ( $p_{H^+}$  4.5—6.0) *Melica uniflora*, *Milium effusum* and *Asperula odorata* have their greatest frequency, while *Hordeum europaeum*, *Mercurialis perennis*, *Allium ursinum*, *Aegopodium podagraria*, *Brachypodium silvaticum*, *Ficaria verna*, *Geum urbanum*, *Anemone hepatica* and *Sanicula europaea* have their greatest frequency in slightly acid, neutral and alkaline soil.

In table 27 is moreover for each  $p_{H^+}$  class stated the average of the number of the species found in the localities examined as also the average of their density. As was the case with the meadows both the number of the species as their density are greatest on habitats with neutral-slightly alkaline soil ( $p_{H^+}$  7.0—7.4), and the number of the species and their density is decreasing with the increasing hydrogen ion concentration of the soil; likewise both the number of the species as the density are lower in the very alkaline soil ( $p_{H^+}$  7.5—7.9) than in the neutral-slightly alkaline one.

Finally it has been examined as regards the single species how the percent number of occurrences with frequency 50 and more than 50 is distributed in the 9  $p_{H^+}$  classes. The result will ap-

pear from table 28, which has been elaborated in the same way as table 16<sup>1)</sup>).

It is not possible to make a determination of the hydrogen ion concentration of wood soil by means of the vegetation after the method mentioned for the meadows, because the ground formations of the forests of beech-trees are too poor on species. On several of the localities examined only a single species is found — as will be seen from the tables — and it is impossible more closely to determine the hydrogen ion concentration by means of a single species; this will be easily understood when glancing at table 27.

On 44 of the localities examined investigations have been made on the course of the transformation processes of nitrogen in the soil. The results hereof as aforementioned will be found in the tables 18—26 and also in the following table 29, where the localities are arranged according to the decreasing hydrogen ion concentration of the soil. It will be seen that the nitrification is wanting or is extremely slight in the most acid soils, i. e. the raw humus soils, consequently the raw humus soil plants are for the greater part obliged to cover their consumption of nitrogen with ammonia, of which rather considerable quantities are formed in the raw humus. In several of the raw humus soils examined at least as large quantities of ammonia nitrogen are formed as of nitrate nitrogen formed in the more nitrifying soils. Thus in all the examined wood soils, even in the most acid ones ample quantities of inorganized nitrogen compounds are formed, so that wood soil plants hardly anywhere, not even on the raw humus soil may suffer from want of nitrogen. Besides it will be seen from table 29 that the greater quantities of ammonia — just as was the case with the meadow soils — are found in the most acid soils, the smallest quantities in the alkaline ones, in which the nitrification passes off most completely.

As will be seen from the preceding, the raw humus soil localities take up an exceptional position as regards the course of the transformation processes of nitrogen, as the nitrification is wanting. Regarding the other localities a closer connection be-

<sup>1)</sup> A table on the number of occurrences without regard to the frequency of the single species in the 9  $pH$  classes, i. e. corresponding to table 15, has been elaborated, but is not published here, as it does not give any essentially different idea of the distribution from that given in table 28.

Table 29. The quantity of nitrogen in the wood soils.

Locality no.	pH	per cent. H <sub>2</sub> O	In 1 kilo dry soil was found mg NO <sub>3</sub>		In 1 kilo dry soil was found mg NH <sub>4</sub>		Locality no.	pH	per cent. H <sub>2</sub> O	In 1 kilo dry soil was found mg NO <sub>3</sub>		In 1 kilo dry soil was found mg NH <sub>4</sub>	
			at once	after one month	at once	after one month				at once	after one month	at once	after one month
79	3.5	40	0	0	10	51	161	5.3	19	9	240	5	30
80	3.5	38	0	0	4	107	167	5.4	32	5	242	20	69
82	3.6	45	0	0	10	60	175	5.5	22	3	202	6	40
88	3.8	25	0	0	2	38	176	5.5	28	0	117	5	12
91	3.9	20	1	1	3	12	181	5.6	21	11	89	8	17
92	3.9	28	1	1	4	10	194	6.0	16	21	114	5	3
93	4.0	15	0	0	1	10	195	6.0	18	23	100	5	3
94	4.0	29	0	0	27	82	196	6.0	26	8	215	5	2
95	4.0	33	0	0	14	70	199	6.1	30	24	89	5	3
99	4.1	38	0	190	7	212	204	6.2	31	0	269	9	12
100	4.1	25	0	0	2	30	208	6.3	19	14	112	1	2
104	4.2	51	0	13	6	105	219	6.5	30	70	222	3	3
105	4.2	40	0	2	99	150	223	6.6	21	6	185	6	4
106	4.2	22	0	0	2	40	226	6.7	28	40	310	3	3
107	4.2	26	1	2	4	31	230	6.8	25	20	111	1	2
116	4.5	21	25	382	6	16	246	7.4	24	15	122	2	1
117	4.6	26	13	385	11	57	251	7.5	35	19	109	1	1
121	4.6	26	10	412	12	16	256	7.6	32	12	152	1	1
129	4.7	22	15	188	3	2	257	7.6	28	23	152	0	0
141	5.0	20	37	209	7	9	258	7.6	26	20	92	0	0
146	5.1	23	12	95	5	5	263	7.8	28	23	240	0	1
157	5.2	23	0	191	6	7	264	7.8	26	31	70	4	2
							265	7.8	25	10	96	0	0

tween the composition of the vegetation and the course of the transformation processes of nitrogen cannot be pointed out.

Finally as mentioned above a determination of the quantities of potassium, phosphate and calcium soluble in distilled water saturated with carbonic acid has been made in soil samples from 12 of the localities examined. These analysis have been grouped in table 30 according to the hydrogen ion concentration of the soil samples.

Table 30.

Locality no. ....	82	104	121	161	175	194	199	208	223	246	251	257
pH .....	3.6	4.2	4.6	5.3	5.5	6.0	6.1	6.3	6.6	7.4	7.5	7.6
K (mg in 1 kilo dry soil)	25	28	24	17	15	33	46	24	30	114	83	12
PO <sub>4</sub> - - - - -	0.3	0.2	2.0	2.0	2.0	3.0	8.0	12.0	4.0	4.0	0.2	16.0
Ca - - - - -	27	25	42	40	45	79	125	207	102	1275	2311	1747

As will be seen, no fixed relation is found between the quantities of potassium and phosphate and the hydrogen ion concentration; on the other hand it appears that the more acid the soils are, the less calcium will on an average be found in the soils. The results of the analysis besides do not imply any closer relation between the composition of the vegetation and the contents of the soil of mineral plant nutrients soluble in water saturated with carbonic acid, calcium however excepted.

Finally I want to mention some investigations of beech-tree forests where the ground vegetation is wanting. Great tracts of Grib Forest, especially the middle parts round Grib Sea and Maglemose bear old beech-tree forests, the soil of which in most places is devoid of vegetation. The light which reaches the soil amounts to 3—5 per cent. of the free daylight; this quantity of light cannot be said to be so small as not to allow the existence of a ground vegetation. In these areas the soil is a very loose and soft raw humus, the hydrogen ion concentration of which measured in soil samples from 10 different localities was found to be from 3.7—3.8, expressed in p<sub>H</sub>. No nitrification takes place in these soil, but on the other hand a very active formation of ammonia; the 10 soil samples examined contained after having stood for one month in the Laboratory no nitrate, but



ample ammonia (from 30—60 mg calculated as  $\text{NH}_4$  per one kilo dry soil). The reason of the wood soil being devoid of vegetation is presumably due to the high hydrogen ion concentration of the soil in connection with want of light, as the high hydrogen ion concentration does not allow the species standing more shade among the mould plants to grow here, while want of sufficient light in general will keep away the raw humus plants which commonly demand more light. Where the forest in single spots is lighter or where the forest adjoins cut areas, meadows and the like, a ground vegetation of raw humus plants will be found, especially formed by *Deshampsia flexuosa*, *Vaccinium myrtillus* and moss species.

### Alteration of the Hydrogen ion Concentration of the Soil when the Forest is cut.

In the following I shall briefly mention some investigations made on the alteration in the abietic raw humus when the forest is cut. All the investigations have been made in Grib Forest, where the soil below the older spruce-forest always is covered by a layer of raw humus about 5—10 cm thick. As a rule the soil is without vegetation, which of course principally is due to want of light; in some cases it may, however, where the plantation is not so dense be covered by an extremely luxuriant moss carpet, for the greater part consisting of *Scleropodium purum*, *Hylocomium splendens* and *H. Schreberi*, as is the case in locality no. 269 (table 31). As will be seen from table 31<sup>1)</sup>, the raw humus is very acid ( $\text{p}_{\text{H}^+}$  3.4—3.6) under the red pine plantations, and no nitrification takes place in it, but on the other hand a very considerable formation of ammonia.

When the spruce-forest is cut, a dense growth of *Senecio silvaticus* (locality nos. 272—278, table 31, see also fig. 3) appears the very first summer. At the same time, however, an alteration takes place in the raw humus, this becoming loose and crumbling, and the hydrogen ion concentration — as will be seen from the table — has decreased very much from  $\text{p}_{\text{H}^+} = 3.5$  to about 5.0

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<sup>1)</sup> The  $\text{p}_{\text{H}^+}$  values indicated in table 31 are electrometrically measured; this also holds good of the  $\text{p}_{\text{H}^+}$  values in table 32 and 33.

and an active formation of nitrate<sup>1)</sup> begins. In a single soil sample (from locality no. 275) so much nitrate was formed in the course of one month that, calculated as  $\text{NO}_3$  it amounts to 1 g in one kilo dry soil. In spite of the very great quantities of nitrate



Fig. 3. Locality no. 273. Grib Forest. *Senecio silvaticus* (with ripe fruits) on the soil of a cut spruce-forest. To the right in the front *Rubus idaeus* is seen. (Phot. 20/8 1917).

formed in these soils the nitrification of the nitrogen must be said to be incomplete, as only about half of the ammonia nitrogen is transformed into nitrate. Furthermore it will appear that as far as the few analysis allow a certain conclusion, considerably greater quantities of easily soluble mineral plant nutrients are found in the cut soil than in the soil below the spruce-forest. It is hardly possible to say anything certain of the reason of the quick de-

<sup>1)</sup> The Swedish investigator Hesselman has likewise substantiated that an active nitrification takes place in the soil after cutting of pine forest. (Hesselman 1917).

Table 31. Analysis of the soil of cut spruce-forest and its vegetation (Grib Forest).

	Dense spruce-forest					1—2 years after the cutting							3—4 years after the cutting				4—5 years after the cutting		
	3-4	3-5	3-6	3-6	3-6	5-6	5-2	5-0	5-1	4-8	4-9	4-9	4-4	4-3	4-7	4-4	4-5	3-8	3-8
pH of the soil sample .....	267	268	269	270	171	272	273	274	275	276	277	278	279	280	281	282	283	284	285
Locality no. ....																			
<i>Deschampsia flexuosa</i> .....	3	3	3	3	3	3	3	20	3	30	3	10	3	3	3	20	100	100	100
<i>Rubus idaeus</i> .....	3	3	3	3	3	3	10	3	3	3	3	50	100	40	20	100	3	3	3
<i>Chamenerium angustifolium</i> .....	3	3	3	3	3	3	30	20	10	3	3	3	30	100	100	80	100	10	3
<i>Senecio silvaticus</i> .....	3	3	3	3	3	100	100	100	100	100	100	100	10	3	3	3	10	3	3
<i>Juncus conglomeratus</i> .....	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	10
<i>Luzula pilosa</i> .....	3	3	3	3	3	3	3	3	3	10	10	30	3	3	3	3	3	3	3
<i>Carex pilulifera</i> .....	3	3	3	3	3	3	3	10	3	3	3	40	3	3	3	10	10	3	3
<i>Rumex acetosella</i> .....	3	3	3	3	3	3	3	30	3	3	10	3	3	3	3	3	20	10	3
<i>Calluna vulgaris</i> .....	3	3	3	3	3	3	3	3	3	3	3	3	10	3	3	20	3	3	20
<i>Vaccinium myrtillus</i> .....	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	30
<i>Galium harycnicum</i> .....	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	40
per cent H <sub>2</sub> O .....	70	53	69	51	56	51	54	50	69	65	57	29	3	66	22	3	3	50	43
NO <sub>3</sub> at once .....	0	0	0	0	0	42	9	143	30	210	56	42	3	105	2	3	3	0	0
— after one month .....	0	0	0	0	0	157	552	778	1290	882	420	366	3	192	51	3	3	0	0
NH <sub>4</sub> at once .....	8	30	10	4	15	10	104	98	105	100	117	37	3	4	5	3	3	4	10
— after one month .....	115	147	139	112	232	10	214	193	355	227	189	56	3	15	30	3	3	42	63
K .....	3	18	3	21	3	3	42	3	3	3	3	39	3	3	3	3	3	3	3
PO <sub>4</sub> .....	3	0.1	3	3	0.1	3	0.5	3	3	3	3	0.4	3	3	3	3	3	3	3
Ca .....	3	25	3	3	23	3	153	3	3	3	3	113	3	3	3	3	3	3	3

In the soil sample

composition of the raw humus after the cutting; perhaps the light plays a part.

As will be seen from table 31, another alteration of the soil takes place during the following years (3—4 years after the cutting), where *Chamaenerium angustifolium* and *Rubus idaeus* are the dominant species, as the hydrogen ion concentration gradually increases while the nitrification at the same time decreases. At this time *Deschampsia flexuosa* as a rule begins to immigrate. 4—5 years after the cutting the hydrogen ion concentration of the soil has nearly reached the same value as in the soil of the spruce-forest. The nitrification has by now ceased completely and the raw humus layer is highly reduced. At that time *Deschampsia flexuosa* is as a rule the dominant species. That the alteration of the soil is not due to the immigration of this species appears from the fact, that the soil from uncovered spots between the tufts of this grass species does not behave otherwise than the soil below the tufts.

The course of development shown in the table has of course not been followed directly in a single locality, but is based on a combination of a number of localities. However, the localities entered in the table as no. 273 and no. 284 are in reality the same localities examined for the first time in 1917 (no. 273), for the second time in the summer 1920 (no. 284). The spruce-forest on this locality was cut in the autumn 1916.

When the beech-forest is cut a similar alteration probably takes place with the hydrogen ion concentration of the soil as described above with regard to the spruce-forest. The results stated in table 32 of analysis made in 1919 on a locality in Ermelunden where an area covered with wood some years before had been cut seem to imply this. In the beech-forest abutting on the cut area the ground vegetation consisted in *Anemone nemorosa* and *Oxalis acetosella*, and the hydrogen ion concentration of the soil expressed in  $p_H$  was found to be 5.2 (locality no. 286). At the bound of the beech-forest and the cut area, where *Mercurialis perennis* was the dominant species, the hydrogen ion concentration of the soil expressed in  $p_H$  had a value of 6.0 (locality no. 287) and on the cut area, where the nettle was dominating, it had a value of 6.6 (locality no. 288). Thus the hydrogen ion concentration was lower in the soil on the cut area than in the soil in the surrounding forest.

Table 32.

	Densq growth	Limit be- tween the growth and the cut area	Cut area
pH <sup>+</sup> of the sample.....	5.2	6.0	6.6
Locality no.....	286	287	288
<i>Anemone nemorosa</i> .....	100	100	»
<i>Oxalis acetosella</i> .....	100	90	»
<i>Mercurialis perennis</i> .....	»	100	»
<i>Geranium Robertianum</i> ....	»	50	50
<i>Geum urbanum</i> .....	»	60	40
<i>Urtica dioeca</i> .....	»	20	100
<i>Milium effusum</i> .....	20	»	»
<i>Rumex nemorosus</i> .....	»	»	10
<i>Ficaria verna</i> .....	»	30	»
<i>Rubus idaeus</i> .....	»	»	40
<i>Scrophularia nodosa</i> .....	»	»	30
<i>Cirsium arvense</i> .....	»	»	20

### Some formations on sunny mineral soil and their habitats.

On sunny mineral soil I have made analysis of formations and habitats in ten different localities. The results of the analysis will be found in table 33, arranged according to the decreasing hydrogen ion concentration of the soil. The three first mentioned localities are from heaths in Jutland. The formation in the first mentioned locality (no. 289), and old west Jutich heath covered with a thick layer of dry peat (raw humus) and underlying bleached sand and hard pan, is that poorest on species and is found on the most acid soil (pH<sup>+</sup> = 3.6). The two following localities (nos. 290 and 291) are hilly heaths near Skorpung. The soil in these heaths is of a more mouldy nature and below the heather is a layer of bleached sand which is only slightly developed, while the hard pan is completely wanting. As will be seen from the table the soil in these localities is somewhat less acid and the number of the species and their density are somewhat greater than is the case as regards the before mentioned west Jutich heath. (In locality no. 291 both the number of the species and their density are considerably greater than in

**Table 33.** Analysis of formations and habitats

Locality no. 289 is situated in a heath near Ulfborg in the western part of Jutland, nos. 290 burned in 1915; the investigation took place in 1919), no. 292 on the Eremitageslette Thisted, no. 295 in the Have valley near Skorping, no. 296 on a slope near Blaakilde in the an open plane in Allindelille Fredskov. The soil in the four last

pH of the soil sample .....	3.6	3.9	4.2	4.7	5.2	6.2	7.4	7.5	7.7	7.7
Locality no. ....	289	290	291	292	293	294	295	296	297	298
<i>Calluna vulgaris</i> .....	100	100	60	60	»	»	»	»	»	»
<i>Empetrum nigrum</i> .....	10	80	»	»	»	»	»	»	»	»
<i>Vaccinium vitis idaea</i> .....	»	70	70	»	»	»	»	»	»	»
<i>Deschampsia flexuosa</i> .....	»	10	50	»	»	»	»	»	»	»
<i>Vaccinium myrtillus</i> .....	»	10	80	»	»	»	»	»	»	»
<i>Arnica montana</i> .....	»	»	90	»	»	»	»	»	»	»
<i>Festuca ovina</i> .....	»	»	»	100	100	»	80	30	40	»
<i>Agrostis tenuis</i> .....	»	»	»	90	100	»	»	»	»	»
<i>Sieglingia decumbens</i> .....	»	»	»	60	»	»	»	»	»	»
<i>Veronica chamaedrys</i> .....	»	»	»	50	20	»	»	10	»	10
<i>Achillea millefolium</i> .....	»	»	»	90	100	100	20	60	60	80
<i>Avena pratensis</i> .....	»	»	»	»	60	»	»	»	»	»
<i>Anthoxanthum odoratum</i> .....	»	»	»	40	100	»	»	»	»	»
<i>Galium verum</i> .....	»	»	»	30	100	80	»	»	30	80
<i>Dactylis glomerata</i> .....	»	»	»	»	»	100	»	100	100	30
<i>Festuca rubra</i> .....	»	»	»	»	»	100	50	100	100	100
<i>Agrostis alba</i> .....	»	»	»	»	»	60	»	»	»	»
<i>Rumex acetosa</i> .....	»	»	»	»	20	50	»	»	50	»
<i>Hypericum perforatum</i> .....	»	»	»	»	»	100	30	»	10	20
<i>Plantago lanceolata</i> .....	»	»	»	»	40	80	»	»	20	40
<i>Origanum vulgare</i> .....	»	»	»	»	»	100	»	»	10	40
<i>Campanula glomerata</i> .....	»	»	»	»	»	100	»	60	50	»
<i>Carlina vulgaris</i> .....	»	»	»	»	»	50	40	30	»	»
<i>Carex glauca</i> .....	»	»	»	»	»	20	80	80	100	100
<i>Festuca elatior</i> f. <i>pseudololiacea</i> ..	»	»	»	»	»	»	60	100	100	»
<i>Avena pratensis</i> .....	»	»	»	»	»	»	80	60	»	»
<i>Koeleria pyramidata</i> .....	»	»	»	»	»	»	100	»	100	»
<i>Poterium sanguisorba</i> .....	»	»	»	»	»	»	100	50	20	»
<i>Medicago lupulina</i> .....	»	»	»	»	»	»	90	50	50	50

from ten localities with sunny mineral soils.

and 291 in heath east of Fræer Purker near Skørping (the heather on locality no. 291 near Hjortekær, no. 293 in a plane in Jægersborg deer-park, no. 294 in Silstrup hills near Buderup valley (Skørping), no. 297 in an old sea-bluff at Klitgaard near Nibe and no. 298 in mentioned localities essentially consists of calcium carbonate (chalk).

Ph <sup>r</sup> of the soil sample .....	3.6	3.9	4.2	4.7	5.2	6.2	7.4	7.5	7.7	7.7
Locality no. ....	289	290	291	292	293	294	295	296	297	298
<i>Brunella vulgaris</i> .....	»	»	»	»	»	»	90	100	20	30
<i>Briza media</i> .....	»	»	»	»	»	»	30	60	100	100
<i>Linum catharticum</i> .....	»	»	»	»	»	»	»	100	30	16
<i>Plantago media</i> .....	»	»	»	»	»	»	40	50	80	»
<i>Calamintha acinos</i> .....	»	»	»	»	»	»	40	100	100	»
<i>Gentiana uliginosa</i> .....	»	»	»	»	»	»	30	50	»	»
<i>Galium mollugo</i> .....	»	»	»	»	»	»	10	100	»	»
<i>Valeriana officinalis</i> .....	»	»	»	»	»	»	»	60	»	»
<i>Poa pratensis</i> .....	»	»	»	»	10	»	»	»	100	»
<i>Primula veris</i> .....	»	»	»	»	»	»	»	40	60	»
<i>Galium boreale</i> .....	»	»	»	»	»	»	»	»	50	20
<i>Cirsium acaule</i> .....	»	»	»	»	»	»	30	30	80	90
<i>Brachypodium silvaticum</i> .....	»	»	»	»	»	»	»	»	»	90
<i>Anemone hepatica</i> .....	»	»	»	»	»	»	»	»	»	100
<i>Polygala vulgare</i> .....	»	»	»	»	»	»	»	»	»	80
<i>Fragaria vesca</i> .....	»	»	»	»	»	»	»	40	10	70
<i>Ononis repens</i> .....	»	»	»	»	»	»	40	20	40	100
<i>Lotus corniculatus</i> .....	»	»	»	»	»	»	40	30	30	50
<i>Pimpinella magna</i> .....	»	»	»	»	»	»	»	»	»	80
<i>Selinum carvifolium</i> .....	»	»	»	»	»	»	»	20	»	100
<i>Campanula rotundifolia</i> .....	»	»	»	»	30	»	»	»	30	50
<i>Valeriana dioeca</i> .....	»	»	»	»	»	»	»	»	»	100
<i>Scabiosa columbaria</i> .....	»	»	»	»	»	»	»	»	»	80
<i>Centaurea jacea</i> .....	»	»	»	»	»	»	»	20	20	60
<i>Leontodon hispidus</i> .....	»	»	»	»	»	»	»	»	»	80
<i>Botrychium lunaria</i> .....	»	»	»	»	»	»	»	10	»	»
<i>Luzula campestris</i> .....	»	»	»	»	20	»	»	»	»	»
<i>Carex panicea</i> .....	»	»	10	»	»	»	»	»	»	»

(To continue p. 90-97.)

Table 33

pH* of the soil sample .....	3.6	3.9	4.2	4.7	5.2	6.2	7.4	7.5	7.7	7.7
Locality no. ....	289	290	291	292	293	294	295	296	297	298
<i>Carex pilulifera</i> .....	»	10	10	»	»	»	»	»	»	»
— <i>caryophyllea</i> .....	»	»	»	»	20	»	»	»	»	»
— <i>digitata</i> .....	»	»	»	»	»	»	40	10	»	»
— <i>hirta</i> .....	»	»	»	»	10	»	»	»	»	»
<i>Poa compressa</i> .....	»	»	»	»	»	»	20	»	»	»
<i>Molinia coerulea</i> .....	»	»	10	»	»	»	»	»	»	»
<i>Holcus lanatus</i> .....	»	»	»	»	10	40	»	»	»	»
<i>Deschampsia caespitosa</i> .....	»	»	»	»	»	»	»	20	»	»
<i>Avena pubescens</i> .....	»	»	»	»	»	»	»	»	»	10
<i>Cynosurus cristatus</i> .....	»	»	»	10	40	»	»	»	»	»
<i>Phleum pratense</i> .....	»	»	»	»	»	30	»	»	»	»
<i>Majanthemum bifolium</i> .....	»	»	40	»	»	»	»	»	»	»
<i>Rumex acetosella</i> .....	»	»	»	20	»	»	»	»	»	»
<i>Melandrium album</i> .....	»	»	»	»	»	»	»	»	20	»
<i>Ranunculus acer</i> .....	»	»	»	»	»	»	»	»	40	»
— <i>bulbosus</i> .....	»	»	»	20	30	»	»	»	»	»
<i>Anemone nemorosa</i> .....	»	»	»	»	»	»	»	»	»	20
<i>Arabidopsis Thaliana</i> .....	»	»	»	»	»	10	»	»	»	»
<i>Mercurialis perennis</i> .....	»	»	»	»	»	»	»	»	»	10
<i>Helianthemum nummularium</i> ....	»	»	»	»	»	»	»	»	»	10
<i>Polygala amarellum</i> .....	»	»	»	»	»	»	20	40	»	»
<i>Parnassia palustris</i> .....	»	»	»	»	»	»	»	»	»	30
<i>Potentilla erecta</i> .....	»	»	30	30	»	»	»	»	»	»
<i>Geum rivale</i> .....	»	»	»	»	»	»	»	»	»	10
<i>Rubus saxatilis</i> .....	»	»	»	»	»	»	»	»	»	40
<i>Agrimonia eupatoria</i> .....	»	»	»	»	»	10	»	»	»	»
<i>Anthyllis vulneraria</i> .....	»	»	»	»	»	»	20	30	»	10
<i>Vicia cracca</i> .....	»	»	»	»	»	»	»	10	30	40
<i>Lathyrus pratensis</i> .....	»	»	»	»	»	20	»	»	»	10
<i>Trifolium repens</i> .....	»	»	»	»	30	»	»	»	»	»



Continuation.

No. of the soil sample .....	3.6	3.9	4.2	4.7	5.2	6.2	7.4	7.5	7.7	7.7
Locality no. ....	289	290	291	292	293	294	295	296	297	298
<i>Trifolium medium</i> .....	✓	✓	✓	✓	✓	✓	30	10	✓	✓
<i>Genista anglica</i> .....	10	✓	20	✓	✓	✓	✓	✓	✓	✓
<i>Pimpinella saxifraga</i> .....	✓	✓	✓	✓	✓	✓	40	✓	40	10
<i>Heracleum spondylium</i> .....	✓	✓	✓	✓	✓	✓	✓	20	✓	10
<i>Daucus carota</i> .....	✓	✓	✓	✓	✓	40	30	✓	✓	✓
<i>Trientalis europaea</i> .....	✓	10	✓	✓	✓	✓	✓	✓	✓	✓
<i>Linaria minor</i> .....	✓	✓	✓	✓	✓	✓	✓	40	✓	✓
<i>Alectorolophus crista galli</i> .....	✓	✓	✓	✓	✓	✓	✓	✓	✓	30
<i>Thymus chamaedrys</i> .....	✓	✓	✓	✓	✓	✓	✓	✓	✓	40
<i>Valeriana excelsa</i> .....	✓	✓	✓	✓	✓	✓	✓	✓	✓	20
<i>Succisa pratensis</i> .....	✓	✓	✓	✓	✓	✓	✓	30	30	20
<i>Centaurea scabiosa</i> .....	✓	✓	✓	✓	✓	✓	✓	✓	40	✓
<i>Fussilago farfarus</i> .....	✓	✓	✓	✓	✓	20	✓	20	✓	✓
<i>Chrysanthemum leucanthemum</i> ..	✓	✓	✓	✓	✓	✓	✓	✓	40	✓
<i>Cineraria integrifolia</i> .....	✓	✓	✓	✓	✓	✓	✓	✓	20	✓
<i>Senecio Jacobaea</i> .....	✓	✓	✓	✓	✓	20	✓	✓	✓	✓
<i>Hieracium pilosella</i> .....	✓	✓	10	10	20	✓	20	10	✓	✓
Number of species .....	3	7	12	13	19	20	28	39	37	44
Density of species .....	1.2	2.9	4.8	6.1	8.6	11.3	13.0	18.0	18.5	21.6
In the soil sample {	per cent H <sub>2</sub> O .....	12	16	14	20	21	✓	15	13	17
	NO <sub>3</sub> at once .....	0	0	0	0	0	✓	30	8	30
	— after one month .....	0	0	0	0	14	✓	41	10	35
	NH <sub>4</sub> at once .....	0	0	0	3	4	✓	2	4	0
	— after one month .....	0	1	2	10	12	✓	2	3	2
	K .....	17	20	✓	✓	✓	✓	27	26	11
	PO <sub>4</sub> .....	0.1	0	✓	✓	✓	✓	0.2	0.3	0.2
Ca. ....	20	16	✓	✓	✓	✓	✓	1911	2035	2277
										2021

locality no. 290. but this is partly due to the fact that the vegetation after heath-fire four or five years ago has not yet come into equilibrium with the surroundings.

No nitrification takes place in the three heath soils examined and it will be seen, that the formation of ammonia is extremely small. Likewise very small quantities of phosphate and potassium are found here. The heath soil therefore is very poor on plant nutrients, what besides is a wellknown matter.

The formations following the heath formations in the table show parallel with the decreasing hydrogen ion concentration of the soil increasing number of the species and their density that culminate in the four mentioned formations which all are found on calcareous soil.

As will be seen, comparatively small quantities of nitrate and ammonia are formed in the soil on open mineral soil, far less than in the wood-soils and the meadow-soils which, I suppose, is due to the poorness of the sunny mineral soils on organic moulding substances. In the soil from the last mentioned locality is a sunny calcareous plane in Allindelille Fredskov which is very poor on mould, in which on the whole no nitrate has been shown. In this plane as also in other similar ones in Allindelille and Kastrup Forest I have repeatedly selected soil samples, but I have never been able to substantiate even the slightest nitrification. Only very small quantities of ammonia and phosphate are found. These soils which almost exclusively consist of calcium carbonate (chalk) are thus extremely poor on plant nutrients and in this respect resemble the heath soil very much. They bear a vegetation very rich on species as also the density of the species is very great. Owing to the poorness on plant nutrients<sup>1)</sup> the single plants are not highly developed and the annual production of matter of the plants is only inconsiderable. Despite the poorness on plant nutrients the vegetation as regards the species found, their number and density, is very different from those of the very acid soils that also are poor on nutrients. This does not imply that the different contents of plant nutrients of the soils

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<sup>1)</sup> The said calcareous planes in Allindelille Forest and Kastrup Forest have surely never born forests, nor can they be expected to do so, as the few wood-trees which are sowing themselves always are decaying and finally fade away. The reason hereof is without doubt the great poorness of the soil on plant nutrients.

plays a greater part as a plant distributing factor than the hydrogen ion concentration. According to some authors the contents of plant nutrients of the soil is said to vary regularly with the variations of the hydrogen ion concentration the most acid soils being poorest on plant nutrients, the neutral and basic soils being the richest. As will be seen from the preceding, this rule is not without exceptions.

### **Recapitulation of the results of the investigations.**

It has been substantiated at the investigations treated in this section, that the hydrogen ion concentration in natural Danish soils lies between the limits 3.4 and 8.0 expressed in  $p_H$  and that the composition of the vegetation depends on the hydrogen ion concentration of the soil, as the single species only are found on soil the hydrogen ion concentration of which lies within a certain range of hydrogen ion concentration characteristic for each single species. Within this range is another narrower range in which the species has its greatest average frequency. As regards the meadows a conclusion may be drawn from the composition of the vegetation to the hydrogen ion concentration of the soil. Furthermore it has appeared that both the number of the species as their density on an average are greatest on soil the hydrogen ion concentration of which lies near the neutral point. With the increasing hydrogen ion concentration of the soil both the number of the species as their density as a rule decrease. On very alkaline soil the number of the species and their density are on an average somewhat lower than in the neutral soil. This soil, therefore, seems to be the most favourable for the growth of most species, while the very acid soil seems to be the most unfavourable.

Thus the hydrogen ion concentration of the soil plays an essential part as a plant distributing factor. As mentioned in the introduction, it may however be a question of both direct and indirect effects hereof. As regards the direct ones the stress has — as mentioned above — been laid on three different effects, i. e. 1) the influence of the hydrogen ion concentration on the contents of mineral plant nutrients of the soil, 2) its influence on the course of the transformation processes of nitrogen, especially the nitrification, as this is said to be more active in neutral

and alkaline soils, weak or wanting in the very acid ones, 3) its influence on the solubility of the aluminum compounds of the soil.

As mentioned above the very acid soils are generally considered as those poorest on mineral plant nutrients, while the neutral and alkaline soils are considered to be the richest hereon. It will appear from the investigations that this as a general rule is not correct, for although the most acid soils have shown to be poor on mineral plant nutrients especially on phosphate, the very alkaline soils, i. e. the soils essentially consisting of calcium carbonate, may be extremely poor on these nutrients. If it was not the different hydrogen ion concentration of the soils examined but their different contents of plant nutrients, which was the reason of their different vegetation, the acid soil poor on nutrients must resemble the alkaline soils poor on nutrients with regard to their vegetation, but this is absolutely not the case.

It will furthermore appear from the investigations that there does not exist in general any closer connection between the intensity of the nitrification and the hydrogen ion concentration of the soil, and that considerable quantities of nitrate may even be formed in very acid soils. In the acid soils more ammonia nitrogen will as a rule be found than in the neutral and alkaline ones, in which the whole quantity of nitrogen liberated quickly is passed into nitrate. Only in the most acid soils, i. e. in the high moor peat, in the heath soils and in most raw humus soils the nitrification does not take place. In the two first mentioned soils the nitrogen is virtually not transformed, as only minimal amounts of ammonia are formed. In the high moor peat and in the heath soil the nitrogen — as besides pointed out by Müller (1918) -- is found in organic bound condition and therefore must be considered as being nearly inaccessible for the higher plants. It is otherwise with the raw humus soils, for although as a rule no nitrification takes place in these soils a formation of ammonia takes place, a formation which even in many cases is considerable. With regard to the raw humus plants it is, therefore, possible, that these plants essentially owe their appearance on the raw humus to the fact that these plants either demand ammonia compounds or at any rate better than other plants are able to use these compounds and that this special ability of the raw humus plants to a higher degree than their sensibility to the hydrogen ion concentration of the soil is the

reason of their occurrence on the raw humus. However, it will appear from the experiments which will be mentioned in the following section, that the nitrate nitrogen and ammonia nitrogen are of the same value as a source of nitrogen both for the alkaline soil plants as for the acid soil plants (including raw humus plants).

Apart from the above mentioned very acid not nitrifying soils it has not been possible with regard to the other soils to show any connection between the intensity of the nitrification and the plant distribution. Thus the investigations made suggest that either it must be the different hydrogen ion concentration of the soils or the degree of solubility dependent on the hydrogen ion concentration of the aluminum compounds of the soil that is a reason of the differences of the vegetation that have been found, in case there are no other factors which vary regularly with the hydrogen ion concentration of the soil than those mentioned above.

It will appear from the culture experiments described in the following section that an essential significance for the plant distribution directly must be attributed to the hydrogen ion concentration. It will also appear that the plants found on acid and alkaline soil respectively generally do not react differently to aluminum compounds in solution.

— — —

## B. CULTURE EXPERIMENTS.

**The growth of the plants in growing media of different hydrogen ion concentrations.**

In the literature only few investigations have been published on the growth of the plants in growing media of different hydrogen ion concentrations. A number of older investigations (Heald 1896, Kahlenberg and True 1896, Hartwell and Pember 1907, Breazeale and Le Clerc 1912, Dachnowski 1914, Miyake 1914) on the growth of the plants in liquids containing different quantities of acid and base are only of small interest, as no regard is paid to the hydrogen ion concentration and its possible alterations during the experiments, that often were made with pure acid or basic solutions without plant nutrients. Of late years Hoagland (1917) has by means of water culture experiments examined the growth of barley in liquids of different hydrogen ion concentration<sup>1</sup>). At the experiments which were made with young plants and which only covered a period of a fortnight, it appeared that the plants were growing equally and very well in nutrient liquids, the  $p_H$  value of which was 5, 6 and 7, while the plants could not stand nutrient liquids with a  $p_H$  value of 3.5 and lower values, nor was it possible to make the plants develop in liquids the  $p_H$  value of which was 8.4.

Investigations have also been made by Joffe (1920 II) who cultivated alfalfa in soil the hydrogen ion concentration of which was varied; the different hydrogen ion concentrations were artificially brought about by means of sulphuric acid and calcium carbonate. He found that the growth of alfalfa was decreasing with the increasing hydrogen ion concentration of the soil. In soils with a  $p_H$  value lower than 4.8 the growth was highly checked.

What is of special interest, however, in connection with the investigations mentioned in this treatise on the significance of the hydrogen ion concentration of the soil as a plant distributing factor is the question, whether the different species reach

<sup>2</sup> In order to avoid precipitations in the basic nutrient liquids Hoagland omitted calcium, magnesium and iron of all the nutrient liquids on the ground that the plants for a shorter experimental period might be able to procure sufficient quantities of these substances from the seeds.

their most vigorous growth at hydrogen ion concentrations characteristic of the different species. From investigations made by Maxwell (1898) on the Sandwich Islands it seems to appear that different culture plants unequally stand high acid concentrations. Maxwell namely found that the ability of the different culture plants to grow in soil watered with citric acid in solution was not the same. Thus alfalfa, barley, wheat, white mustard and rape succumbed if the soil, in which they grew, was watered with citric acid of 0,1 per cent, while maize and especially pearl millet (sorghum) stood this treatment very well. The two latter species also appeared to be the only ones thriving in the very acid soils, which according to Maxwell are found in many places in the Sandwich Islands.

Finally a paper has been published by Salter and Mc Ilvaine (1920) on the growth of some culture plants in nutrient liquids of different hydrogen ion concentrations. The two authors came to the conclusion that maize reached its best growth in nutrient liquids, the  $p_H$  value of which was 5, while wheat, soya-beans and alfalfa were thriving best in nutrient liquids, the  $p_H$  value of which was 6. Thus maize seems to favour a higher hydrogen ion concentration than the three other species.

The object of my experiment was to examine, whether the species especially found on very acid soil (acid soil plants) are thriving best in growing media of higher hydrogen ion concentration than the species found especially in slightly acid, neutral and alkaline soil (alkaline soil plants). For these experiments I therefore partly selected acid soil plants, partly alkaline soil plants, but of course only such species as are growing comparatively quickly as the experiments could not cover more than one period of growth.

Besides Danish indigenes a single culture plant, i.e. barley (*Hordeum distichum*) was included in the experiments, which must be considered as an alkaline soil plant, as it only reaches a satisfactory development in arable soil giving an alkaline or a neutral reaction.

**Experiments on the growth of different species in natural soil of different hydrogen ion concentration.** During the summer 1918 a series of experiments was made. The alkaline soil plants *Tussilago farfara* and *Poterium sanguisorba* and the acid soil plants *Senecio silvaticus* and *Deschampsia*

*flexuosa* were cultivated in 7 different soils, the hydrogen ion concentrations of which were known and varied over the  $pH$  range 3.6—7.7.

The soils originated with 7 of the localities mentioned in the previous section, from which very large soil samples had been carried home. A part of each of these soil samples was used for the soil analysis, the results of which will be found in the tables 18 (loc. no. 82), 21 (loc. no. 167), 24 (loc. no. 226), 26 (loc. no. 257), 31 (loc. no. 273) and 33 (loc. no. 292 and 298). The results of the analysis are also stated in table 34. For convenience the soils are here designated with the letters (a—g) stated at the top of the table. Below the letters is stated the number of the locality from which the soil samples originated.

The soils were put in flower-pots each holding about 2 litres of soil. The pots were then placed in the open air and were daily watered with distilled water. The plants to be experimented on were sown in the flower-pots on the 23rd of April. *Tussilago* however not until the 16th of May<sup>1</sup>). Each of the 4 species were sown severally in the 7 different soils, thus I had 4 series of experiments, one for each species, and as each series was doubled, the total number of flower-pots thus was 56. As soon as the plants had developed, a thinning was made, care being taken that the number of remaining plants of each species was the same in all the flower-pots.

The plants of *Deschampsia flexuosa* were growing very badly in soil e, f and g (see fig. 4), and in these soils they were highly chlorotic from the very beginning. In the 4 other soils the plants had a pure green colour, in the two most acid soils even a deep dark green colour<sup>2</sup>).

*Senecio silvaticus* and *Deschampsia flexuosa* grew extremely badly in nearly neutral and alkaline soils (e, f and g, see fig. 5). In soil g, calcareous soil poor on mould from Allindelille Fred-

<sup>1</sup> The material of seed sown was as regards *Deschampsia flexuosa* and *Senecio silvaticus* gathered the previous year in Grib Forest, as regards *Poterium sanguisorba* near Skörping, also the previous year, and as regards *Tussilago* it was gathered the same spring in Vangede. The fruits of *Tussilago* are ripe in the first half of May; then they can germinate but already at the end of May they loose for always the power of germination.

<sup>2</sup> In soil b the growth of the plants was — as will be seen from fig. 4 — poorer than in soil a and c. This without doubt was due to the fact that soil b is much poorer on accessible nitrogen compounds than the other two soils.



Fig. 4—7 show the growth of 4 different species in 7 soils with different hydrogen ion concentrations. The flower-pots with the most acid soils are standing to the left, those with the alkaline soils to the right.



Soil	a	b	c	d	e	f	g
pH	3.6	4.7	5.2	5.4	6.7	7.6	7.7

Fig. 4. *Deschampsia flexuosa*. Phot. <sup>15</sup>/<sub>8</sub> 1918.



Soil	a	b	c	d	e	f	g
pH	3.6	4.7	5.2	5.4	6.7	7.6	7.7

Fig. 5. *Senecio silvaticus*. Phot. <sup>30</sup>/<sub>7</sub> 1918.



Soil	a	b	c	d	e	f	g
pH	3.6	4.7	5.2	5.4	6.7	7.6	7.7

Fig. 6. *Poterium sanguisorba*. Phot. <sup>15</sup>/<sub>8</sub> 1918.



Soil	a	b	c	d	e	f	g
pH	3.6	4.7	5.2	5.4	6.7	7.6	7.7

Fig. 7. *Tussilago farfarsus*. Phot. <sup>16</sup>/<sub>9</sub> 1918.

skov, the plants succumbed even quite after having developed only two yellow cotyledons. In the soils c and d *Senecio silvaticus* reached its best growth; its colour was here pure green. In the most acid soil (a) most of the plants gradually died. *Senecio silvaticus* thus cannot stand as acid soil as *Deschampsia flexuosa*.

*Tussilago farfars* and *Poterium sanguisorba* grew best in basic and nearly neutral soils (see fig. 6 and 7), and the growth decreased proportionally with the increasing hydrogen ion concentration of the soil. The colour of the plant was in all cases normally green, in the most acid soils, however, somewhat darker than in the other soils<sup>1</sup>).

As regards *Senecio* the experiments were finished on the 30th of July, as regards *Deschampsia* and *Poterium* on the 15th of August, and as regards *Tussilago* on the 16th of September. The plants were then weighed, first in fresh condition, afterwards dried. The results will be seen from table 34. Each of the numbers stated off the names of the species is the weight of the plants developed in a single flower-pot (average of the plants of two interdependent flower-pots).

It will appear from the results of the experiments that the plants pre-eminently found on very acid soil on an average are thriving best in very acid soils, those preferentially found on neutral-alkaline soils in the neutral-alkaline soils. It is a matter of course that the growth does not vary regularly with the hydrogen ion concentration of the soil, nor was this to be expected, as the soils in question also differed with regard to other factors than the hydrogen ion concentration, thus e. g. as regards the nitrification energy. The growth, however, seems more dependent on the hydrogen ion concentration than on the nitrification energy. *Senecio silvaticus*, which according to Hesselman (1917) is a nitrate plant, thrives best, it is true, in the soil in which the greatest quantity of nitrate is found, viz. soil c, but as will be seen its growth in the rather acid soil d is considerably better

<sup>1</sup> As will be seen from fig. 6 the growth of *Poterium* was smaller than in soil f, although the hydrogen ion concentration of these two soils was nearly the same. This must be supposed to be due to poorness of soil g on plant nutrients, especially on nitrogen. When no difference was found with regard to *Tussilago* in soil f and g, it may be due to this plant not needing as much nitrogen as *Poterium*.

Table 34.

The growth of 4 species in 7 soils with different hydrogen ion concentrations.

Soil .....		a	b	c	d	e	f	g
The soils originate with locality number		82	292	273	167	226	257	298
Analysis of the soil	PH.....	3.6	4.7	5.2	5.4	6.7	7.6	7.7
	NO <sub>3</sub> at the drawing of samples .....	0	0	9	5	40	23	0
	NO <sub>3</sub> after one month....	0	0	552	242	310	152	0
	NH <sub>4</sub> at the drawing of samples .....	10	3	104	20	3	0	1
	NH <sub>4</sub> after one month....	60	10	214	69	3	0	2
	K .....	25	»	42	»	»	12	21
	PO <sub>4</sub> .....	0.3	»	0.5	»	»	16	0.2
	Ca .....	27	»	153	»	»	1747	2021
Deschampsia flexuosa. Weight (g), fresh.		37.4	23.2	<b>68.3</b>	60.2	15.8	8.3	5.4
— — — — —, dry..		6.5	4.1	<b>12.6</b>	10.0	2.8	1.5	1.0
Senecio silvaticus. Weight (g), fresh ...		0.3	8.5	<b>29.0</b>	14.5	5.3	5.2	0
— — — — —, dry ....		0.1	1.2	<b>4.8</b>	2.4	0.9	0.8	0
Poterium sanguisorba. Weight (g), fresh.		0	2.0	9.1	14.3	25.8	<b>27.0</b>	14.1
— — — — —, dry ..		0	0.4	1.9	3.0	5.5	<b>5.5</b>	3.5
Tussilago farfara. Weight (g), fresh....		2.5	12.0	22.1	39.8	46.4	<b>50.2</b>	50.0
— — — — —, dry .....		0.3	1.9	3.4	6.0	7.1	<b>7.2</b>	7.0

than in the less acid soil e, though a more active nitrification takes place in the latter soil, than in soil d. Furthermore the different nitrification energies of the different soils do not seem to influence the thrift of *Tussilago* and *Poterium*.

It holds good of all the plants experimented on that the more acid soil they were growing in, the darker were their leaves.

**Experiments on the growth of different species in nutrient liquids with different hydrogen ion concentrations.** The first experiments were made during the summer 1917, and were made on the acid soils plants *Deschampsia flexuosa*, *Senecio silvaticus*, *Sphagnum rubellum*, *S. magellanicum*, *S. apiculatum*

and *S. subsecundum* and on the alkaline soil plants *Tussilago farfars* and *Hordeum distichum*<sup>1)</sup> as also on the aquatic plant *Helodea canadensis*.

At these experiments it was a question of keeping the hydrogen ion concentration of the nutrient liquids constant during the period of experiment. As it was not possible to add any buffer substances in greater quantities to the nutrient liquids because their salt concentration might be higher than the plants could stand, a "running-water-culture" was used, i. e. the plants were cultivated under such conditions that the nutrient liquids incessantly were renewed.

As the plants were to be cultivated in nutrient liquids of 6 different hydrogen ion concentrations 6 carboys each holding 60 litres of nutrient liquids were placed in the green house in a proper height. On each carboy a glass tube was placed acting as a siphon, from which the nutrient liquid was conducted to the different culture glasses through a glass distributor. By means of intercalated rubber-tubes provided with pinch-cocks the supply of the liquid could be regulated. The nutritive liquids were lead to the surface of the culture glasses, while a self-regulating glass siphon from the bottom of the glasses lead the surplus nutrient liquid away. The supply of nutrient liquid was regulated so that the liquid in the glasses was renewed about 3 times in the 24 hours.

For the preparation of the nutrient liquids tap water was used. One litre of the prepared nutrient liquid contained:

0.075 g  $\text{KNO}_3$   
 0.035 -  $\text{MgSO}_4, 7\text{H}_2\text{O}$   
 0.050 -  $\text{CaCl}_2, 6\text{H}_2\text{O}$   
 0.025 -  $\text{KH}_2\text{PO}_4$

besides the substances<sup>2)</sup> originally found in the tap water. Besides the quantity of hydrochloric acid or sodium hydroxyde solution required to give the nutrient liquid the desired hydrogen ion concentration, was added to each of the 6 nutrient liquids.

When the nutrient liquids in the carboys were used, new

<sup>1</sup> The material of seed sown originating with a "pure line" of Goldthorpe barley was kindly supplied by Professor W. Johannsen.

<sup>2</sup> The tap water contained in one litre 0.18 g Ca and much iron that it was superfluous to add a ferric salt to the nutrient liquid.

nutrient liquids were prepared by introducing in the empty carboys the single nutritive salts as concentrated solutions together with the quantity of acid or base necessary for each nutrient liquid; then the carboys were filled with tap water. The nutrient liquid was carefully mixed by conducting a strong current of air; then its hydrogen ion concentration was colorimetrically measured and possibly corrected by adding a little hydrochloric acid or sodium hydroxyde solution. Besides the hydrogen ion concentration was also controlled in the culture glasses regularly during the period of the experiment.

The six different nutrient liquids severally had the following  $p_{H^+}$  values: 3.5; 4.5; 5.5; 6.5; 7.5; 8.0.

The *Sphagnum* species and *Helodea* were cultivated in cylindric glasses holding 0.5 litre, the other plants in wide-necked intransparent glass bottles, also holding 0.5 litre. The plants were placed in the glassees on perforated cork-stoppers where they were fastened by means of glass cotton.

*Deschampsia flexuosa*, *Hordeum distichum*, *Senecio silvaticus* and *Tussilago farfara* were made to germinate in sand and having reached a suitable size they were placed in the culture glasses. Of *Deschampsia flexuosa* 10 plants were placed in each glass.

Of *Helodea* about two g of fresh shoots were placed in each glass, of the submerged *Sphagnum subsecundum* 3 g, of *S. rubellum* and *S. magellanicum* 5 g, and of *S. apiculatum* 15 g. The three latter non-submerged species were placed in the glass, so that the top of the shoots were on a level with the brim of the glass and rose about 2 cm above the surface of the liquid. The shoots of the 5 last mentioned species were gathered in Nature.

Double series of all the experiments were made. The cultivation in water culture began as regards all the species on the 22nd of May.

During the period of the experiment it held good of all the species, *Helodea* however expected, that hardly any growth took place in the two alkaline nutrient liquids, in which the plants quickly became chlorotic, while the *Sphagnum* plants in these two nutrient liquids quickly died. In the slightly acid nutrient liquid, the  $p_{H^+}$  value of which was 6.5, the two acid soil plants *Deschampsia flexuosa* and *Senecio silvaticus* quickly became

chlorotic and only grew very slowly, while *Tussilago* and *Hordeum* in the same nutrient liquid remained pure green and reached a vigorous growth<sup>1)</sup>.

I held good of all the species that when cultivated in the most acid nutrient liquids in which they could thrive they assumed a darker green colour than in the less acid ones.

Moreover it must be stated that the plants during the first fortnight of the period of the experiment did not show as great differences in the growth in the different nutrient liquids as later. *Tussilago* and *Hordeum* even grew somewhat more at the beginning in the nutrient liquid the  $p_H$  value of which was 5.5 than in that with a  $p_H$  value of 6.5, while the case was nearly the reverse later. At the first stages of development the plants thus comport somewhat otherwise than later; consequently when only using a fortnightly period for the experiments — as was made by the said American investigator (Hoagland 1917, Sal-

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<sup>1)</sup> Stohmann (1862) already observed that the higher plants, most aquatic plants however excepted, do not thrive in an alkaline nutrient liquid, in which they quickly will become chlorotic. The reason of the plants becoming chlorotic when growing in a nutrient liquid giving an alkaline reaction is generally considered to be that the plants cannot absorb the iron, because it is in a precipitated condition. Gile and Carrero (1916) have also substantiated that chlorotic rice plants cultivated in a nutrient liquid giving alkaline reaction contained less iron than rice plants cultivated in acid nutrient liquids.

Besides I have not been able in any of the nutrient liquids used at the above mentioned experiments, not even in the alkaline ones, to observe the least trace of deposit. Therefore it is not immediately obvious that the chlorosis of the plants in the alkaline nutrient liquids is due to the fact that the iron is not accessible, because it is in a precipitated condition. If the reason of the chlorosis is that the iron is found in a precipitated condition, it is difficult to understand that the acid soil plants become chlorotic in a nutrient liquid, the  $p_H$  value of which is 6.5, while the alkaline soil plants do not become chlorotic in such a nutrient liquid, for if the acid soil plants cannot absorb the iron, because it is found in a precipitated condition, the alkaline soil plants must be able to do so, as they do not become chlorotic in a nutrient liquid, the  $p_H$  value of which is 6.5. The matter cannot be explained by assuming that the alkaline soil plants may put up with less iron than the acid soil plants, for, as mentioned on p. 5 alkaline soil plants generally contain as much iron as the acid soil plants.

Gile and Carrero (1916) state that it is possible to cultivate plants in an alkaline nutrient solution so as partly to avoid chlorosis, viz. in the case when iron is given as tartrate, in which case it is not precipitated because it enters in a complex ion. Experiments which I have made with barley plants, do not corroborate the correctness of this statement.

Table 35.

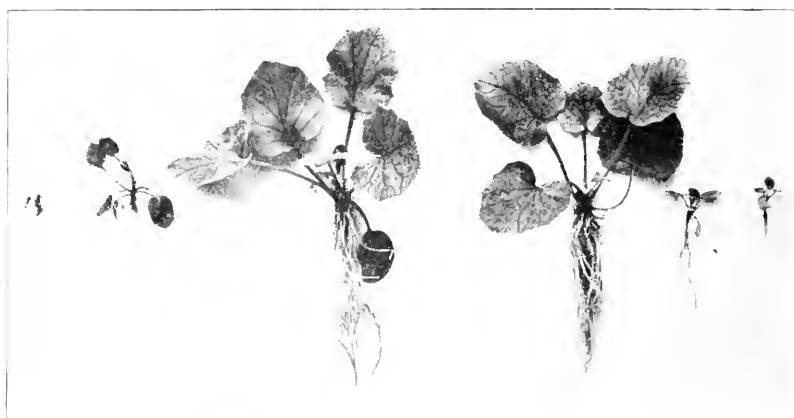
The growth of different plants of different hydrogen ion concentration. The numbers state the weight of the plants in g at the end of the experiments.

pH values of the nutrient liquids	3.5	4.5	5.5	6.5	7.5	8.0
<i>Sphagnum rubellum</i> , fresh.....	<b>22.0</b>	14.0				
— — , dried .....	<b>1.8</b>	1.3			dead	
<i>Sphagnum magellanicum</i> , fresh.....	<b>18.0</b>	12.0				
— — , dried .....	<b>1.7</b>	1.0			dead	
<i>Deschampsia flexuosa</i> (10 plants), fresh .....	4.0	<b>4.5</b>	3.0	1.7		
— — (10 — ), dried .....	0.6	<b>0.7</b>	0.5	0.3		dead
<i>Senecio silvaticus</i> (1 plant), fresh .....	21.0	<b>25.0</b>	15.0	5.0	1.0	
— — (1 — ), dried .....	2.8	<b>3.2</b>	2.0	0.6	0.2	dead
<i>Sphagnum apiculatum</i> , fresh .....	31.0	<b>35.0</b>	20.0			
— — , dried .....	3.3	<b>4.0</b>	2.1			dead
<i>Sphagnum subsecundum</i> , fresh .....	4.3	5.2	<b>6.0</b>	4.3		
— — , dried .....	0.3	0.5	<b>0.6</b>	0.4		dead
<i>Hordeum distichum</i> (1 plant), fresh .....	3.0	45.0	86.0	<b>88.0</b>	10.0	4.0
— — (1 — ), dried .....	0.4	6.3	13.0	<b>13.0</b>	1.4	0.6
<i>Tussilago farfara</i> (1 plant), fresh .....	dead	7.0	30.0	<b>35.0</b>	2.0	1.0
— — (1 — ), dried .....		1.1	4.0	<b>4.2</b>	0.3	0.2
<i>Helodea canadensis</i> , fresh .....			2.6	3.3	4.4	<b>6.4</b>
— — , dried .....	dead		0.2	0.3	0.4	<b>0.7</b>

ter and Mc Ilvaine 1920), other results will be reached than when using a longer period.

All the experiments were finished on the 21st of July, thus after a period of about two months. The plants were weighed after the expiration of the experiments, first in fresh condition, later in dried condition. The result will be seen from table 35. The plants from the duplicate cultures were in good conformity as regards the weight, consequently this is stated in the table as the average value. It will be seen from the table that the diffe-

rent species reached their most vigorous growth at very different hydrogen ion concentrations. *Deschampsia flexuosa* and *Senecio silvaticus* which in Nature only are found in very acid soils, reached their best growth in the nutrient liquid, the  $pH$  of which was 4.5, the alkaline soil plants *Tussilago farfara* (see fig. 8) and *Hordeum distichum* in the nutrient liquid, the  $pH$  value of which was 6.5. None of the plants with which experiments have been made could thrive in alkaline nutrient liquids, the aquatic



$pH$  3.5      4.5      5.5      6.5      7.5      8.0

Fig. 8. The growth of *Tussilago farfara* in nutrient liquids of different hydrogen ion concentration. Phot. 21/7 1917.

plant *Helodea canadensis* however excepted. The latter has even its most vigorous growth in the most alkaline of the nutrient liquids, and as will be known it is also found in Nature in very calcareous, i. e. alkaline waters. It is besides — as mentioned above (p. 110 the foot-note) a well-known case that aquatic plants contrary to other plants can thrive in alkaline nutrient liquids.

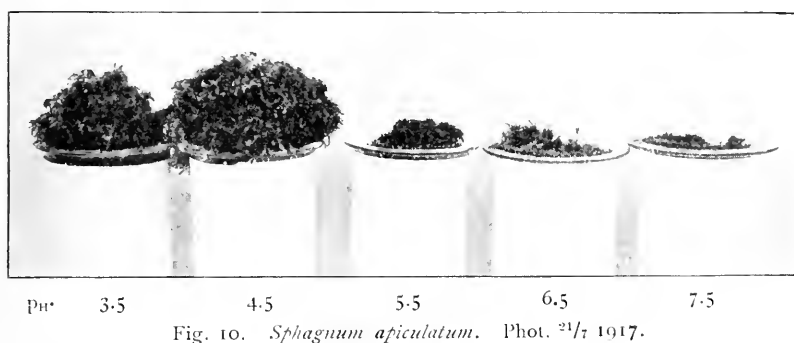
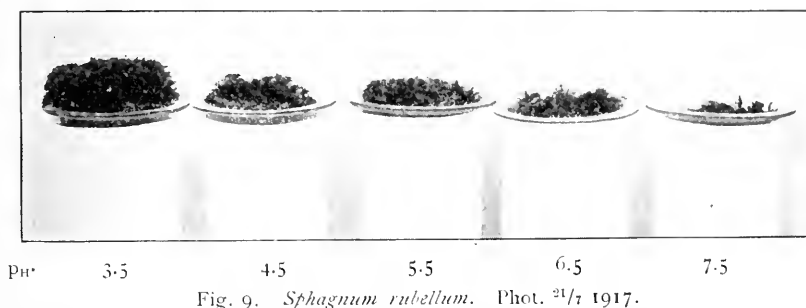
The *Sphagnum* species were able to thrive in nutrient liquids, the hydrogen ion concentration of which lies within narrow limits (see fig. 9 and 10). *S. rubellum* and *S. magellanicum* are — as will be known — specific high moor plants and in conformity herewith they were thriving best in the most acid liquids; *S. apiculatum*, which as a rule in Nature is found in less acid growing substratum than the two first mentioned species, were thriving best in nutrient liquids the  $pH$  values of which



was 4.5. Finally the submersed *S. subsecundum* showed its best thriving in nutrient liquids the  $p_{H^+}$  value of which was 5.5. The plants of this species originated with Store Grib So, the water of which has a hydrogen ion concentration of 5.3 expressed in  $p_{H^+}$ .

During the summer 1920 another series of experiments was made with *Deschampsia flexuosa*, *Senecio silvaticus* and *Tussilago*

Fig. 9—10 show the growth of two different *Sphagnum* species in nutrient liquids of different hydrogen ion concentrations.



*farfarus*. A running water culture was, however, not used, but they were placed as is generally the case with water culture experiments, with the only different that during the whole period a constant current of atmospheric air was conducted through each single nutrient liquid. This "airing" of the nutrient liquids appeared to have an extremely beneficial influence on the growth of the plants, and hereby was moreover obtained that the carbonic acid disengaged at the root respiration was removed.

During the period of the experiments the hydrogen ion con-

centrations of the nutrient liquids were measured every second day and, if necessary, corrected by adding hydrochloric acid or sodium hydroxyde solution. In this way the hydrogen ion concentration was kept constant in the different liquids. Besides the nutrient liquids were renewed once during the period of the experiments.

As nutrient liquid was used boiled and filtered tap water to which was added to each litre:

1.12 g  $\text{NaNO}_3$   
 0.30 -  $\text{NH}_4\text{Cl}$   
 0.25 -  $\text{MgSO}_4, 7\text{H}_2\text{O}$   
 0.25 -  $\text{CaSO}_4, 2\text{H}_2\text{O}$   
 0.25 -  $\text{KCl}$   
 0.25 -  $\text{KH}_2\text{PO}_4$   
 0.01 -  $\text{FeCl}_3$

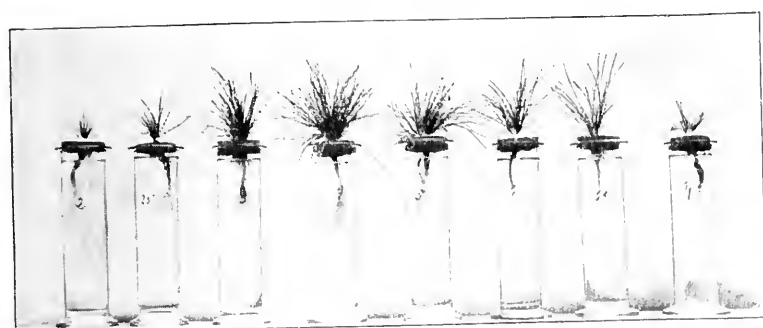
beside the quantity of hydrochloric acid or sodium hydroxyde solution necessary to give the solutions the desired hydrogen ion concentration. Nutrient liquids were used, the hydrogen ion concentration of which expressed in  $\text{pH}$ , varied from 2—7.5. It will be seen from table 36 at what hydrogen ion concentrations the single species were cultivated. All the cultures were made in duplicate. After having germinated in moist sand the plants were placed in the culture glasses on the 1st of June. The ex-

**Table 36.**

The growth of 3 different species in nutrient liquids of different hydrogen ion concentrations. The numbers state the weight of the plants in g at the end of the experiment.

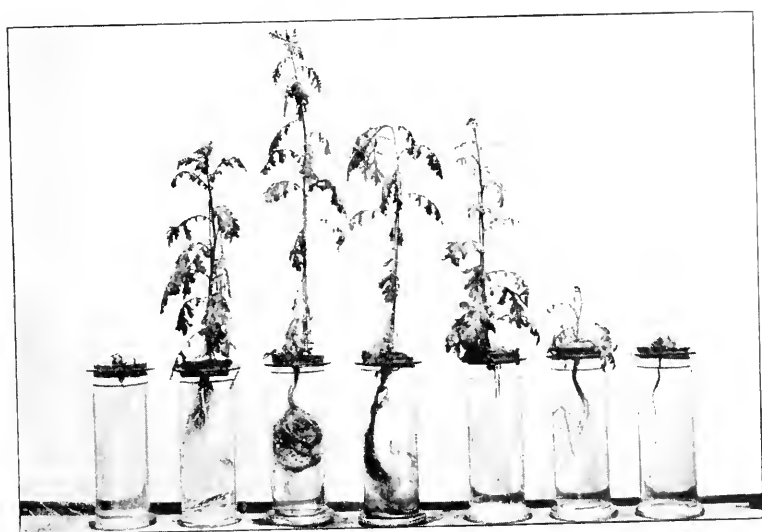
$\text{pH}$ values of the nutrient liquids	2.0	2.5	3.0	3.5	4.0	4.5	5.0	6.0	6.5	7.0	7.5
<i>Deschampsia flexuosa</i> (10 pl.), fresh	0.4	1.0	3.5	»	<b>5.2</b>	»	4.0	2.1	1.8	0.4	»
— — (10 —), dry.	0.1	0.2	0.6	»	<b>0.8</b>	»	0.6	0.3	0.3	0.1	»
<i>Senecio silvaticus</i> (1 plant), fresh			1.5	18.0	<b>26.0</b>	»	18.0	14.0	4.2	1.3	»
— — (1 —), dry.			0.3	2.2	<b>3.4</b>	»	2.3	2.0	0.5	0.2	»
<i>Tussilago farfara</i> (1 plant), fresh.				0.6	6.2	8.7	10.3	<b>20.5</b>	»	<b>20.0</b>	0.4
— — (1 —), dry.				0.1	0.8	1.2	1.4	<b>2.5</b>	»	<b>2.5</b>	0.1

Fig. 11—13 show the growth of different species in nutrient liquids of different hydrogen ion concentrations.



pH. 2.0 2.5 3.0 4.0 5.0 6.0 6.5 7.0  
The plants are chlorotic

Fig. 11. *Descampsia flexuosa*. Phot.  $\frac{1}{8}$  1920.



pH. 3.0 3.5 4.0 5.0 6.0 6.5 7.0  
The plants are chlorotic

Fig. 12. *Senecio silvaticus*. Phot.  $\frac{1}{8}$  1920.



pH. 3.5 4.0 4.5 5.0 6.0 6.5 7.0 7.5

Fig. 13. *Tussilago farfara*. Phot.  $\frac{1}{8}$  1920.

periment was finished on the 1st of August (see fig. 11, 12 and 13) when the plants were weighed first in fresh condition, later dried. The result will be seen from table 36.

As was the case at the experiment in 1917, the acid soil plants *Descampsia flexuosa* and *Senecio silvaticus* became chlorotic, not only in the alkaline, but also in the slightly acid nutrient liquids, the  $p_{H^+}$  values of which were 6.0 and 6.5 respectively, while the alkaline soil plant *Tussilago farfarnus* did not become

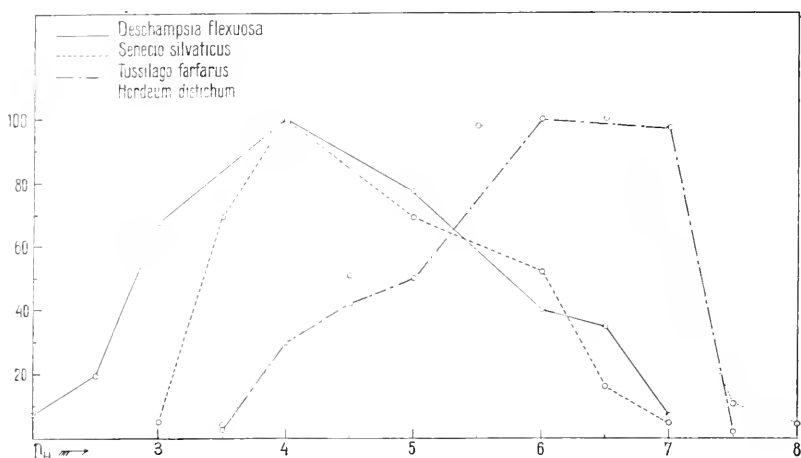


Fig. 14. The relative growth of four different species in nutrient liquids of different hydrogen ion concentrations.

chlorotic in the slightly acid liquids, in which it even reached its most vigorous growth. Besides it will be seen from the table that the results of the experiments are in fair conformity with the corresponding one of 1917, although the nutrient liquids had an essentially different composition and i. a. was remarkable for a far higher salt concentration.

The relative growth of the three species at different hydrogen ion concentrations has been grafically represented in fig. 14, as the highest weight reached for each single species has been equalized to 100 and the other weight figures are converted in relation hereto. In the same figure is stated the relative growth of *Hordeum distichum* calculated at the experiments of 1917. The figure thus represents the growth of two acid soil plants and two alkaline soil plants at different hydrogen ion concentrations. As will be seen, the graphs of the acid soil plants and alkaline soil plants differ very much.

It appeared from all the experiments, as will be seen from the preceding observations, that no land plants, not even alkaline soil plants, can thrive in alkaline nutrient liquids, while the alkaline soil plants on the other hand thrive excellently in alkaline reacting soils and also, as will be seen from the following, in an alkaline reacting sand culture. An explanation is necessary here. When an alkaline soil plant cannot thrive in alkaline reacting liquid, it is a natural conclusion that the plant cannot thrive either in the soil, if the soil liquid round the roots gives an alkaline reaction. If the plant thrives very well in an alkaline reacting soil, i. e. a soil the aquaous extract of which is alkaline, the conclusion may be drawn that the soil liquid round the roots of the plants does not react alkaline, but gives a slight acid reaction. The slightly acid reaction which must be supposed to dominate in the membranes of liquid round the roots, is doubtless brought about by the carbonic acid disengaged at the root respiration, as these membranes of liquid round the roots probably may be nearly saturated with carbonic acid<sup>1</sup>). If alkaline reacting calcareous soil is deposited in water, and if the mixture is saturated with carbonic acid, the hydrogen ion concentration will increase to a value of about 6.0 expressed in  $p_{H^+}$ , this hydrogen ion concentration being extremely favourable to an alkaline soil plant; even at considerably lower tension of carbonic acid the hydrogen ion concentration might easily reach a  $p_{H^+}$  value lower than 7. Besides there is no reason to suppose that alkaline soil plants disengage greater quantities of carbonic acid from the roots than acid soil plants. The latter can without doubt also in calcareous soil increase the hydrogen ion concentration in the liquid round the roots to a value which expressed in  $p_{H^+}$  lies between 6 and 7, but as it will be seen from

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<sup>1</sup>) If the carbonic acid in an alkaline reacting liquid constantly is removed from the surroundings of the roots, e. g. by running water, a bad thriving in connection with chlorosis even in calciphilous plants may be observed also under natural conditions. On a chalk cliff at Blaakilde near Skorping where *Tussilago farfara* and *Urtica dioica* were growing, the water at several places leaked out from the cliff. The very calcareous water had a neutral reaction ( $p_{H^+}$  = about 7.0) where oozing directly out of the cliff and the plants, that grew here showed a normal green colour, but at some distance from these places both *Tussilago* and *Urtica* were found to be chlorotic, and the water which incessantly run over the soil and the roots showed an alkaline reaction ( $p_{H^+}$  = 7.5—8.0) having gradually given off part of its carbonic acid.

Table 37.

Summary of the germination of different species in nutrient liquids with different hydrogen ion concentrations.

pH of the nutrient liquids	3.0	3.5	4.0	4.5	5.5	6.5	7.5	8.0
<i>Deschampsia flexuosa</i> . . . . .	germination normal, several root-hairs were developed						germination slow, germ root remains short and without root-hairs	
<i>Arnica montana</i> . . . . .	germination slow, no root-hairs developed	germination normal, several root-hairs were developed					do.	
<i>Senecio silvaticus</i> . . . . .	do.	germination slow, root-hairs were developed	germination normal, several root hairs were developed				germination slow, germ root thin, almost without root-hairs	
<i>Chamaenerium angustifolium</i>	do.	do.	do.				germination slow, root-hairs scarce	
<i>Poterium sanguisorba</i> . . . . .	germ root developed, but immediately turns brown at the point and dies		germination slow, root-hairs scarce	germination normal, several root hairs developed			do.	
<i>Tussilago farfara</i> . . . . .	do.		do.	do.			do.	

the experiments, such a hydrogen ion concentration is too small to have the plants reach a normal growth.

It will appear from what has been stated that the hydrogen ion concentration also in acid soils on account of the secretion of carbonic acid of the roots must be higher in the soil liquid in the immediate surroundings of the roots than in the soil liquid as a whole. The difference, however, must be decreasing with the increasing hydrogen ion concentration of the soil, and at a  $p_H$  value of 4 or lower values the carbonic acid cannot any more exercise any influence on the hydrogen ion concentration.

**Experiments on the germination of different species in liquids with different hydrogen ion concentrations<sup>1</sup>.** In May

<sup>1</sup> Germination experiments in liquids containing different quantities of acid and base have been made by Loew (1903), Fischer (1907), Promsy (1911);

1918 germination experiments were made with *Deschampsia flexuosa*, *Arnica montana*, *Senecio silvaticus*, *Chamaenerium angustifolium*, *Poterium sanguisorba* and *Tussilago farfara* in nutrient liquids of different hydrogen ion concentrations. The germination took place in conical flasks closed with a cotton wool plug. The nutrient liquid had the same composition as that described on p. 108, and in each flask so much was put as to cover the bottom with a layer of the liquid that was 5 mm thick. During the experiment that lasted for about a fortnight, the nutrient liquids were changed daily. Each species was sown in nutrient liquids of 8 different hydrogen ion concentrations, as will be seen from table 37.

The different values of the hydrogen ion concentration did not influence the germination percentage, as nearly all seeds and fruits germinated, but on the other hand the quality of the germination, as will appear from table 37. It will be seen, that the different species have a greater or smaller range of hydrogen ion concentration, in which the germination is normal, and that the acid soil plants are able to germinate normally at higher hydrogen ion concentrations than the alkaline soil plants.

### **Have ammonia salts and nitrates different values as source of nitrogen for acid soil plants and for alkaline soil plants?**

As shown in section A, the greater quantities of ammonia are found in the most acid soils, while only extremely small quantities of ammonia are found in the less acid, in the neutral and in the alkaline soils, as the ammonia nitrogen here quickly is passed into nitrate. As mentioned on p. 11, a number of investigators have, therefore, thought that the species found on very acid soil (acid soil plants) can use ammonia, while those

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Crocker and Davis (1914) and Onodera (1916). It holds good of these experiments as of those mentioned on p. 102 that as no regard is paid to the hydrogen ion concentration they are of very little interest. Germination experiments with maize, wheat, soya-beans and alfalfa in liquids of different hydrogen ion concentrations have been made by Salter and Mc Ilvaine (1920). They found that the said species are able to germinate at somewhat higher hydrogen ion concentration than the plants can stand later during their growth.

found on alkaline soil (alkaline soil plants) require nitrate as the source of nitrogen and cannot use ammonia, which as regards these plants is considered a less favourable source of nitrogen. Experiments, however, have not been made in order to have the theory in question corroborated. On the other hand, there is a rather considerable literature on the value of ammonia nitrogen and nitrate nitrogen as the source of nitrogen for the culture plants<sup>1</sup>).

It appears from this literature that the nitrogen nitrate nearly always has appeared to be a favourable source of nitrogen for the higher plants, while ammonia salts have given very different results; in some cases they have proved useful, in other cases less useful as source of nitrogen, in numerous cases they have even had a very disadvantageous effect. Thus as early as 1868 Hellriegel states that when using ammonia salts as source of nitrogen in his sand cultures he never got normal plants. Hampe (1868) and Beyer (1869) also reached bad results when using ammonia salts as source of nitrogen for plants in water culture; the plants grew rather well at first but died later pretty suddenly. Wagner (1869) reached rather good results with maize in water culture when using ammonia phosphate as a source of nitrogen, while the result when using ammonia carbonate was very bad. Hässelbarth (1878) found that nitrates had a far better effect in sand culture on barley than ammonia salts, which might give good results when marl was added at the same time, the advantageous effect of which Hässelbarth ascribes to its furthering effect on the nitrification. Heiden (1879) has, when using ammonia sulphate as nitrogen fertilizer at field experiments reached worse results than when not giving nitrogen fertilizer at all. Kellner (1884) has shown, that rice is thriving as well with ammonia salts as with nitrates and that this plant in the rice fields cannot have any nitrate whatever as the nitrification does not take place here<sup>2</sup>). Pitch (1887, 1893 and 1896) in order to avoid the nitrification has cultivated barley and oats sterily in sterilized soil both with nitrates and with ammonia salts and reached the best results when using nitrates; oats, however, was

<sup>1</sup> A very elaborate list of literature is found in Wolkoff (1918 II).

<sup>2</sup>) Statements found in several phyto-physiological text-books to the effect that ammonia salts are a favourable source of nitrogen to fen- and moor-plants are surely derived herefrom.



thriving comparatively better than barley when using ammonia salts as nitrogen fertilizer.

The opinion of the reason of the unfavourable effect of ammonia salts is varying. Mayer (1881) draws the attention to the fact that ammonia sulphate and ammonia chloride have a "physiologically acid effect", i. e. that when used as fertilizer they involve that the reaction of the soil is altered in acid direction, which he thinks is due to the plants using the base of the salts ( $\text{NH}_4\text{OH}$ ), while the acids are left in the soil. It is the contrary with nitrate of soda, as the reaction of the soil when this substance is used is altered in an alkaline direction, which he thinks may be explained by the plants in this case using the nitric acid, whereby the base is left in the soil. This substance, therefore, is described as being "physiologically alkaline".

Several authors therefore have been of opinion that the physiologically acid nature of the ammonia salts is the reason of their disadvantageous effect, as the growing medium gradually should become so acid, that the acid reaction has a directly injurious effect on the roots, e. g. Ehrenberg (1908), who found, that plants in sand culture suffered heavily from supply of ammonia phosphate, while they were thriving well when having potassium nitrate. At the end of the experiment it appeared that the culture medium (consisting of sand mixed with a little high moor peat) gave a very acid reaction on the litmus paper in the vessels to which ammonia salts had been added, while the vessels to which potassium nitrate had been added only gave a slightly acid or nearly neutral reaction (however, it must be remembered that the culture medium on account of the high moor peat from the beginning must have given a very acid reaction). In a later paper (1910) Ehrenberg, however, means that the injurious influence of the ammonia salts is due not so much to their physiologically acid quality as to the fact that the vessels used for these experiments nearly always are made of zinc, and this metal according to Ehrenberg is said to be able to liberate the base ( $\text{NH}_4\text{OH}$ ) from the ammonia salts, the hydroxyl ions of which then have a corrodent effect on the plant roots (l. c. p. 129).

That ammonia salts when used as fertilizer cause the quantity of acid in the soil to increase has many times later been

observed, thus by Daikuhara (1914, p. 1), Truog (1918, p. 184), and by Allison and Cook (1917) who state that although to a smaller degree this also takes place when the soil is uncovered, which — they think — is due to the consumption of nitrogen of the microorganisms. Furthermore it has many times been substantiated that heavy soluble phosphoric acid fertilizer when given together with ammonia sulphate becomes more easily accessible for the plants than if ammonia salts are not given at the same time (Priantischnikow 1902, Böttcher 1907, Söderbaum 1908, Mitscherlich and Simmermacher 1913); this is explained, at any rate partly, by the physiologically acid character of the ammonia salts.

Experiments with ammonia salts as source of nitrogen in arable soils have, however, in many cases given rather good results (Lipman and Gericke 1918). The different species react different to ammonia salts (Wolkoff 1918), and the same holds good of the soils, as ammonia salts often on sandy soils give worse results than on clayey ones (Haselhoff 1914). Lipman and Blair (1918), however, mean that ammonia sulphate in all cases is a worse nitrogen fertilizer than nitrate of soda.

Several investigators have thought it possible to explain the less advantageous effects in many cases called forth by ammonia salts not as a consequence of their physiologically acid qualities, but as a consequence of ammonia ions simply being toxic to the plants, thus Allison (1918), Wolkoff (1918 I and II) and Söderbaum (1910, 1915, 1916, 1917, 1918). The last-mentioned investigator has at vessel experiments with a sand soil substantiated decidedly injurious effects called forth by ammonia salts on barley and wheat, while a quantity of nitrate of soda equivalent to the given quantity of ammonia salts used as fertilizer in the same soil gave excellent results. When fertilizing with increasing quantities of ammonia sulphate decreasing yield was obtained with wheat; where larger quantities of ammonia sulphate were used, the yield was smaller than in the vessels to which no nitrogen fertilizer was added. Oats, rye and potatoe were contrary to barley and wheat, as these plants gave as good yield when fertilized with ammonia sulphate as when using nitrate of soda. Different ammonia salts were tried on barley, which all proved injurious to a different degree (Söderbaum 1917). Thus ammonia chloride had the most toxic effect, then

comes arranged after the decreasing toxicity the sulphate, the nitrate, the carbonate and the phosphate. The injurious effect of the salts partly manifested itself in a great chequing of the growth, partly in the leaves, that had developed, quickly becoming brown in the point; they often quite faded later. Based on these observations Söderbaum is of opinion that the ammonia ions have toxic effects, as from reasons to which I shall later revert he thinks, that it cannot be a question of the physiologically acid effects of the salts (Söderbaum 1915, p. 10).

Söderbaum's experiments thus seem to corroborate the above mentioned theory, according to which acid soil plants shall be able to use ammonia nitrogen, what the alkaline soil plants on the other hand cannot do, for the ammonia salts at his experiments proved toxic to the plants which require a limy soil for their good thriving, i. e. the alkaline soil plants (barley and wheat), while ammonia salts were not toxic to the plants thriving well in acid soil (potatoe, rye and oats).

To examine how alkaline soil plants and acid soil plants are thriving, both when having ammonia salts and when having nitrates as source of nitrogen, I made during the summer 1918 a number of sand culture experiments with *Deschampsia flexuosa*, *Eriophorum vaginatum*, *Senecio silvaticus*, *Chamaenerium angustifolium*, *Oxalis acetosella*, *Asperula odorata*, *Mercurialis perennis*, *Poterium sanguisorba* and *Tussilago farfarus*.

The sand that was used at the experiments was strand sand which was washed first with hydrochlorid acid then with tap water, until the water did not give an acid reaction any more. Finally the sand was washed with distilled water and then dried.

The vessels in which the plants were cultivated were cylindrical earthen jars, glazed within, with a bottom area of about 250 cm<sup>2</sup> and holding 5.5 kilos dry sand. Each receiver got the following fertilizer:

1 g CaSO<sub>4</sub>, 2 H<sub>2</sub>O  
1 - CaHPO<sub>4</sub>, 2 H<sub>2</sub>O  
1 - KCl  
0,5 - MgSO<sub>4</sub>, 7 H<sub>2</sub>O  
0,02 - FeCl<sub>3</sub>.

The two first mentioned substances were in powdered condition mixed with the dry sand. The other substances were

added in solution, one half immediately at the beginning of the experiment, the rest 6 weeks later.

All the plants with which experiments were made were cultivated in three different ways, viz. partly with ammonia chloride, partly with ammonia nitrate and partly with sodium nitrate as source of nitrogen. The quantity of nitrogen was in all cases the same. In order to reach the natural conditions as much as possible, the nitrogen was not added at a time, but every week 25 mg of nitrogen was added to the jars, thus

95.4 mg  $\text{NH}_4\text{Cl}$ , 71.4 mg  $\text{NH}_4\text{NO}_3$ , 151.7 mg  $\text{NaNO}_3$

respectively. The nitrogen compounds were added dissolved in the water used at the watering. The cultures remained in a green house and during the period of the experiments the plants were watered with distilled water not containing copper.

In the cultures in which ammonia salts were used as source of nitrogen, the nitrification had of course to be prevented. To attain this, an absolute sterile culture which it is extremely difficult to carry through, is not necessary, for as the nitrification bacteria cannot stand drying up (Lafar 1904—1906) they are not found in the air. At a temporary experiment it also appeared that no nitrification takes place in moist sand, to which is added ammonia chloride, when care is taken that the sand does not get into touch with objects, which have been brought into contact with moist soil. To be quite sure that no nitrification of the ammonia nitrogen takes place at the experiment, the sand in the jars to which ammonia chloride was added, was often controlled partly by trying whether nitrate might be shown in the sand by means of diphenylamin sulphuric acid, partly by inoculating Winogradsky's solution (see Lafar 1904—1906, p. 146) with a little of the sand. Traces of nitrate in the sand have never been shown, nor could samples of the sand bring about nitrification in Winogradsky's solution. It may, therefore, be supposed that nitrification during the experiment has not taken place in any of the cultures.

*Deschampsia flexuosa*, *Eriophorum vaginatum*, *Senecio silvaticus*, *Chamaenerium angustifolium* and *Poterium sanguisorba* were sown in the jars on the 15th of April, *Tussilago farfara* on the 8th of May. *Mercurialis perennis*, *Oxalis acetosella* and *Asperula odorata* on the other hand were placed in the sand as under-

ground shoots, which first had been sterilized by aid of a 2 per cent sublimate solution. To give the plants shade enough the culture of the three wood-plants were placed below a tent of green tissue-paper, through which about 7 per cent of the day light might pass.

All the cultures were made in duplicate. In the jars, in which the plants were sown, a thinning was made as soon as the plants had developed, leaving an equal number of plants of each species in each jar. The period of the experiments was, as will appear from table 39 (p. 130), of uneven length for the different species, the experiments with the slowly growing species being extended over a longer period than those with quickly growing ones.

During the period the different species were very different.

At the experiments where the plants got ammonia chloride, the different species comported themselves as follows: *Poterium* and *Tussilago* as early as a week after the germination assumed a deep green colour; about 5 days afterwards they began to fade and finally died after having developed only one or two small leaves. *Mercurialis*, *Asperula*, *Oxalis* and *Chamaenerium* also faded after first having assumed a deep green colour, this however only taking place after 3—4 weeks after the beginning of the experiment. *Senecio* on the other hand grew for a long time and reached a rather considerable development; not until the 27th of June the plants began to fade and finally died. Only *Deschampsia* and *Eriophorum* thrived during the whole period of the experiment.

With ammonia nitrate as source of nitrogen *Deschampsia*, *Eriophorum*, *Senecio*, *Chamaenerium*, *Oxalis* and *Asperula* developed excellently (fig. 15—20). *Mercurialis*, *Poterium* and *Tussilago* also grew well at the beginning, the growth at a given time even being more vigorous than in the cultures in which sodium nitrate was source of nitrogen (see fig. 23), but at the end of July the three said species began to turn very dark green and at the beginning of August they began to fade and finally died (see fig. 21, 22 and 24).

With sodium nitrate as source of nitrogen *Tussilago*, *Poterium* and *Mercurialis*, reached their best growth (fig. 21, 22 and 24), while the other species showed a very slow growth (fig. 15

Fig. 15—24. The growth of different plants in sand culture with ammonia chloride, ammonia nitrate and sodium nitrate respectively as source of nitrogen.

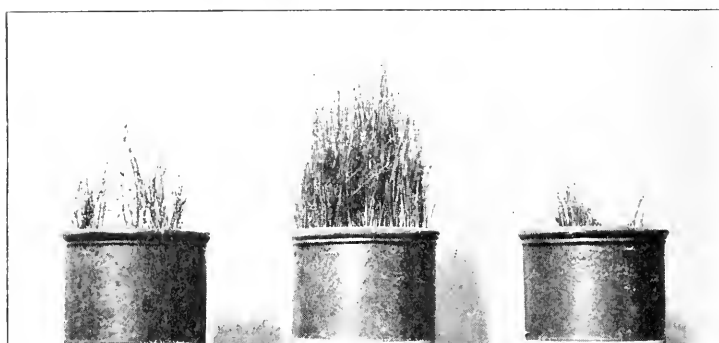


$\text{NH}_4 \text{Cl}$

$\text{NH}_4 \text{NO}_3$

$\text{NaNO}_3$

Fig. 15. *Deschampsia flexuosa*. Phot. 14/7 1918.

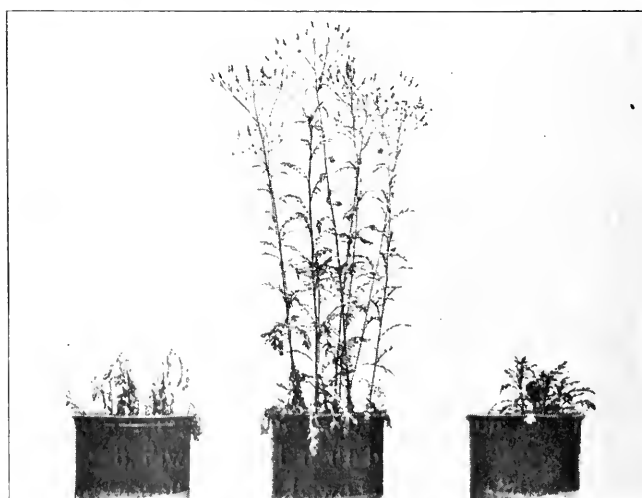


$\text{NH}_4 \text{Cl}$

$\text{NH}_4 \text{NO}_3$

$\text{NaNO}_3$

Fig. 16. *Eriophorum vaginatum*. Phot. 14/9 1918.

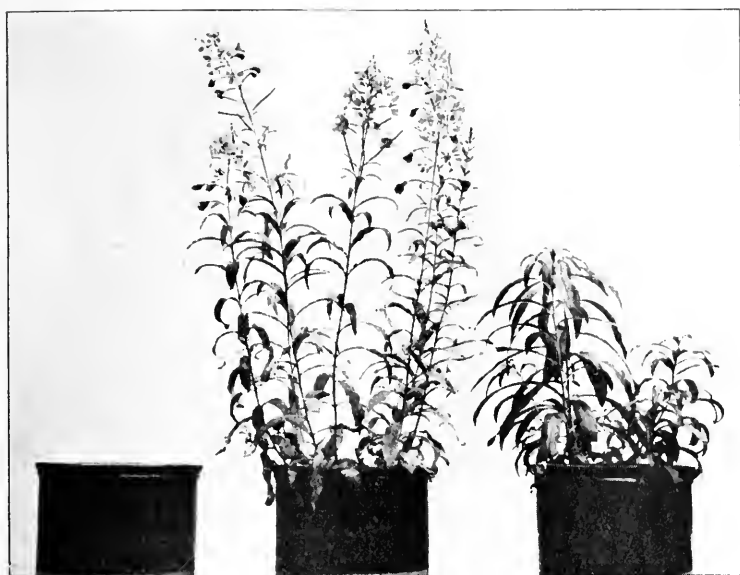


$\text{NH}_4 \text{Cl}$

$\text{NH}_4 \text{NO}_3$

$\text{NaNO}_3$

Fig. 17. *Senecio silvaticus*. Phot. 3/7 1918.



$\text{NH}_4 \text{Cl}$

$\text{NH}_4 \text{NO}_3$

$\text{NaNO}_3$

Fig. 18. *Chamaenerium angustifolium*. Phot. 17/7 1918.



$\text{NH}_4 \text{Cl}$

$\text{NH}_4 \text{NO}_3$

$\text{NaNO}_3$

Fig. 19. *Oxalis acetosella*. Phot. 17/7 1918.



$\text{NH}_4 \text{Cl}$

$\text{NH}_4 \text{NO}_3$

$\text{NaNO}_3$

Fig. 20. *Asperula odorata*. Phot. 17/7 1918.



$\text{NH}_4 \text{Cl}$

$\text{NH}_4 \text{NO}_3$

$\text{NaNO}_3$

Fig. 21. *Mercurialis perennis*. Phot. 17/7 1918.



$\text{NH}_4 \text{Cl}$

$\text{NH}_4 \text{NO}_3$

$\text{NaNO}_3$

Fig. 22. *Poterium sanguisorba*. Phot. 7/8 1918.



$\text{NH}_4 \text{Cl}$

$\text{NH}_4 \text{NO}_3$

$\text{NaNO}_3$

Fig. 23. *Tussilago farfara*. The appearance of the cultures about 1½ month after the beginning of the experiment. Phot. 20/6 1918.



$\text{NH}_4 \text{Cl}$

$\text{NH}_4 \text{NO}_3$

$\text{NaNO}_3$

Fig. 24. *Tussilago farfara*. Same cultures as those seen in fig. 23 but about 2 months later. Phot. 23/8 1918.



Table 38.

The  $p_{H^+}$  values of the nutrient liquids absorbed by the sand. \* at the  $p_{H^+}$  values stated designates that the plants at the times of the determination had died.

Source of nitrogen	$NH_4 Cl$	$NH_4 NO_3$	$Na NO_3$
	$p_{H^+}$	$p_{H^+}$	$p_{H^+}$
<i>Deschampsia flexuosa</i> .....	2.8	3.5	7.5
<i>Eriophorum vaginatum</i> .....	3.2	4.0	8.0
<i>Senecio silvaticus</i> .....	3.0*	4.0	8.2
<i>Chamaenerium angustifolium</i> .....	3.0*	3.9	7.8
<i>Oxalis acetosella</i> .....	3.4*	4.0	8.0
<i>Asperula odorata</i> .....	3.4*	4.2	7.8
<i>Mercurialis perennis</i> .....	3.8*	4.2	8.0
<i>Poterium sanguisorba</i> .....	3.5*	4.0	8.0
<i>Tussilago farfarus</i> .....	3.5*	4.7	8.0

to 20) and quickly became chlorotic. The chlorosis was most marked in *Deschampsia*, *Eriophorum* and *Senecio*.

As evidently it was the physiologically acid reaction of the ammonia salts and the physiologically alkaline one of the sodium nitrate that was dominating, a determination of the hydrogen ion concentration<sup>1)</sup> of the nutrient liquid absorbed by the sand was made in all cultures; for this determination litmus paper, lacmoide paper (see Hempel 1917) and Congo paper were used. The papers were placed in the sand well moistened with distilled water and remained in contact with the sand for about half an hour. Then their colour tone was compared with the tones which were brought about by different standard liquids of known hydrogen ion concentration. The determination was made on the 3rd of July; however, as regards the cultures in which the plants had died previously, immediately after the death of the plants. The result will be seen from table 38, in which the  $p_{H^+}$  values found are stated.

In table 39 are stated the weight of the plants in dry condition<sup>2)</sup> at the end of the experiment as also the duration of the experiment for the single species.

<sup>1)</sup> The nutrient liquid originally had a  $p_{H^+}$  value of 6.

<sup>2)</sup> As part of the plants had faded before taken out of the sand to be weighed, their weight in fresh condition cannot be stated.

Table 39.

The weight of the plants in dry condition at the end of the experiment. The figures designate the weight in g of all the plants from a single jar.

	NH <sub>4</sub> Cl	NH <sub>4</sub> NO <sub>3</sub>	Na NO <sub>3</sub>	Duration of the experiment
<i>Deschampsia flexuosa</i> .....	30.0	46.0	23.0	15/4 — 1/8
<i>Eriophorum vaginatum</i> .....	2.2	7.7	1.3	15/4 — 14/9
<i>Senecio silvaticus</i> .....	5.0	12.1	3.0	15/4 — 3/7
<i>Chamaenerium angustifolium</i> .....	0.0	17.9	11.0	15/4 — 17/7
<i>Oxalis acetosella</i> .....	1.0	2.7	1.6	15/4 — 17/7
<i>Asperula odorata</i> .....	0.0	5.9	1.2	15/4 — 17/7
<i>Mercurialis perennis</i> .....	0.6	8.4	11.2	15/4 — 17/7
<i>Poterium sanguisorba</i> .....	0.0	3.0	10.8	15/4 — 8/8
<i>Tussilago farfatus</i> .....	0.0	15.0	21.8	8/5 — 25/8

It will be clearly seen from the figures in table 38 that the disadvantageous effects of the ammonia chloride are due to its physiologically acid reaction, as the absorption of nitrogen involves that the hydrogen ion concentration of the nutrient liquid increases. The plants die when the hydrogen ion concentration has reached the value critical for the species, the alkaline soil plants first because these plants cannot stand comparatively high hydrogen ion concentration; the acid soil plants on the other hand are more resistant, as these plants can stand very high hydrogen ion concentrations. It must be observed that the plants of *Tussilago* and *Poterium* which died as quite small plants, had not altered the hydrogen ion concentration of the nutrient liquid of the whole quantity of sand, as only the nutrient liquid in the sand from the surface and from the immediate surroundings of the roots showed the high hydrogen ion concentration ( $p_H$  3.5) as stated in table 38, while the nutrient liquid in the sand from the bottom of the jars had their original hydrogen ion concentration ( $p_H$  6).

It appears from table 38, that ammonia nitrate also has a physiologically acid effect, as the plants when having this salt as source of nitrogen also brought about an increase of the hydrogen ion concentration of the nutrient liquid, although this did

not take place as quickly as when using ammonia chloride<sup>1)</sup>. Therefore the plants were thriving for a long time when having ammonia nitrate, but as regards the alkaline soil plants the time at last came at which the hydrogen ion concentration reached the critical limit, when the plants died.

Finally it will be seen from table 38, that sodium nitrate has physiologically alkaline reaction. This is the reason of the alkaline soil plants thriving very well when having sodium ni-



$\text{NH}_4 \text{Cl}$

$\text{Na NO}_3$

Fig. 25. *Tussilago farfara* in sand culture with ammonia chloride and sodium nitrate respectively as source of nitrogen. The sand in both jars contained calcium carbonate (levigated chalk). Phot. 20/9 1919.

trate, while on the other hand acid soil plants, which cannot stand the alkaline reaction, showed bad thriving and became chlorotic.

Thus the experiments in reality only illustrate the different ability of the species to stand high and low hydrogen ion concentrations, but nothing is elucidated as to whether ammonia or nitric acid is of different value to them as source of nitrogen. To have this question elucidated it is necessary to make the

<sup>1)</sup> Based on my experience I cannot say anything as to whether the plants of the ammonia nitrate absorb comparatively more ammonia than nitric acid, but it is not necessary to presume this to explain the physiologically acid reaction of the ammonia nitrate, for it must be born in mind that the other nutrients also are of importance. Apart from the nitrogen, the plants of the other nutrients absorb the greatest quantity as base (Ca, K). If now also half of the nitrogen is given as base, as is the case when ammonia nitrate is the source of nitrogen, the plants presumably of the aggregate nutrient liquid have absorbed more base than acid; consequently the hydrogen ion concentration of the nutrient liquid will increase.

Table 40.

The weight (g) of the plants at the end of the experiments.

	NH <sub>4</sub> Cl + Ca CO <sub>3</sub>	Na NO <sub>3</sub> + Ca CO <sub>3</sub>
<i>Tussilago farfarus</i> , fresh .....	198.0	205.0
— — dry .....	25.5	27.0
<i>Poterium sanguisorba</i> , fresh .....	54.0	55.0
— — dry .....	11.3	12.0

experiments in such a way, that the hydrogen ion concentration in the nutrient liquid is kept constant. In sand cultures this may e. g. be reached by mixing calcium carbonate with the sand. Thus the acid liberated by the ammonia salts will be neutralized. Such an experiment was made in 1919 with *Tussilago farfarus* and *Poterium sanguisorba*. The plants in one experiment got ammonia chloride, in another sodium nitrate and the experiments were executed in the manner described above, with the only difference that the sand in all the jars was mixed with levigated chalk (200 g to 1 kilo sand). The cultivation was commenced on the 1st of June and was finished on the 20th of September. The result will be seen from table 40 and as regards *Tussilago* also from fig. 25. It will be seen that the growth of the plants was almost the same, whether having ammonia chloride or sodium nitrate.

Simultaneously with the sand culture experiment water culture experiments were made with *Tussilago farfarus* and *Deschampsia flexuosa*. Both species were cultivated partly in nutrient liquid containing ammonia chloride (solution a), partly in nutrient liquid containing sodium nitrate (solution b). The two nutrient liquids contained in 1 litre boiled and filtered tap water:

Solution a	Solution b
1.0 g NH <sub>4</sub> Cl	1.6 g Na NO <sub>3</sub>
<hr/>	
0.25 g Ca SO <sub>4</sub> , 2 H <sub>2</sub> O	
0.25 - Mg SO <sub>4</sub> , 7 H <sub>2</sub> O	
0.25 - K Cl	
0.25 - KH <sub>2</sub> PO <sub>4</sub>	
0.01 - Fe Cl <sub>3</sub>	

The nutrient liquids were not renewed during the period of the experiment, which lasted from the 1st of June to the 15th of August. During the whole period a constant current of atmospheric air was conducted through all the nutrient liquids.

At the experiments with *Tussilago* the  $p_{H^+}$  value of the nutrient liquid was kept constant at 6 by constantly adding sodium solution and hydrochloric acid respectively. In two cases *Tussilago*, however, was cultivated in solution *a* without making anything in order to keep the hydrogen ion concentration of the nutrient liquid constant. In these two cases the hydrogen ion concentration of the nutrient liquid increased as the plants grew until a  $p_{H^+}$  value of 3.3, after which the plants died (see fig. 26). The plants growing in the nutrient liquids the hydrogen ion concentration of which was kept constant, developed normally and showed a homogeneous growth, whether growing in solution *a* or in solution *b* (see fig. 26).

At the experiments with *Deschampsia flexuosa* the  $p_{H^+}$  values of the nutrient liquids were kept constant at 4. Also in this case the plants were homogeneously developed whether they grew in solution *a* or in solution *b*. The weight of the plants at the end of the experiment will be seen from the following table 41.

It appears from the last mentioned experiment that both alkaline soil plants and acid soil plants are thriving equally well whether ammonia salts or nitrates are given as source of nitrogen, if only the hydrogen ion concentration of the nutrient liquids is the same and is kept constant. Ammonia ions thus are not toxic for plants, neither for alkaline soil plants nor for acid soil plants. When Söderbaum when using ammonia salts as nitrogen fertilizer on barley and wheat reached very disadvantageous results, it has certainly been due to the physiologically acid reaction of the salts and not, as he thinks, to toxic effects called forth by ammonia ions. When rye, oats and potatoe were not injured, it is without doubt due to these plants being able to stand the increase of the hydrogen ion concentration of the soil, which is produced when the ammonia is absorbed by the plants. Söderbaum (1915, p. 10) thought it possible that the disadvantageous effects might be due to their physiologically acid reaction, but at closer consideration he rejected this explanation, and he stated three arguments against it. The first argument

was, that the toxic effects occurred already while the plants were quite young, and Söderbaum holds that at such an early time the plants cannot have absorbed so much nitrogen that a considerable



$\text{NH}_4 \text{Cl}$

$\text{NH}_4 \text{Cl}$

$\text{NaNO}_3$

Fig. 26. *Tussilago farfurus* in water culture. The plant to the right has got sodium nitrate as source of nitrogen and the  $\text{pH}$ -value of the nutrient liquid has been kept constant at 6.0. The plant in the middle has got ammonia chloride and the  $\text{pH}$ -value of the nutrient liquid has also been kept constant at 6.0. The plant to the left has got ammonia chloride, but nothing is made here to keep the hydrogen ion concentration of the nutrient liquid constant; therefore the plant has altered the hydrogen ion concentration to a  $\text{pH}$ -value of 3.3, which has involved the death of the plant.

able alteration of the reaction might have taken place. As it will appear from the sand culture experiments made by me, a considerable alteration of the hydrogen ion concentration of the nutrient liquid takes place where ammonia salts are used as nitrogen

**Table 41.** The weight (g) of the plants developed in nutrient liquids the hydrogen ion concentration of which was kept constant.

	$\text{NH}_4 \text{Cl}$	$\text{NaNO}_3$
<i>Tussilago farfurus</i> 1 plant, fresh.....	58.0	06.0
— — 1 — , dry .....	6.5	6.3
<i>Deschampsia flexuosa</i> (10 plants), fresh.....	6.0	6.1
— — (10 — , dry .....	1.0	1.0

fertilizer for plants while these are quite young, but this alteration at the beginning only is quite local, viz. in the liquid in the immediate surroundings of the roots and therefore may become very important.

The second argument stated by Söderbaum is, that ammonia nitrate also has a toxic effect, and this salt is according to Söderbaum "physiologically neutral". That ammonia nitrate, however, is physiologically acid will be seen from the experiments made by me. When finally Söderbaum as a third argument states that at his experiments he mixed the soil with bone-dust containing sufficient calcium carbonate to neutralize the acid, that might possibly be liberated at the consumption of nitrogen of the plants, I want to observe that the sandy soil used by Söderbaum beforehand might have been acid, and the calcium carbonate has then been neutralized by the acid found in the soil.

When ammonia chloride at Söderbaum's experiments was the ammonia salt which was most unfavourable to barley plants, while ammonia sulphate and ammonia phosphate had a better effect, it may be due to the fact that the plants of the two last-mentioned salts can use both the acid and the base, while for the greater part they only absorb the base of the ammonia chloride.

Besides it must be noted that when the effects of the ammonia salts at my first experiments were so great that the alkaline soil plants were killed, which was not the case at Söderbaum's experiments, this is essentially due to my cultures not containing buffer. Söderbaum used soil, and soil contains considerable quantities of buffer; furthermore a part of the ammonia nitrogen may have undergone nitrification before being absorbed by the plants.

Söderbaum states that the wheat and barley plants fertilized with ammonia salts contained an abnormally great quantity of nitrogen contrary to the plants fertilized with nitrate of soda.

In order to examine whether the same held good of the plants used for my experiments, I determined the nitrogen percentage<sup>1)</sup> in some of the *Tussilago* plants used at the above described experiments. Only the leaves were analyzed. Normally these leaves contain about 2.26 per cent of nitrogen<sup>2)</sup>. The plants from the sand culture experiments in which the hydrogen ion concentration of

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<sup>1)</sup> Reckoned on the dry substance.

<sup>2)</sup> The normal contents of nitrogen of the leaves was determined by analyzing leaves taken on different localities in Nature. The contents of nitrogen varied from 2.0—2.35 per cent. The average contents of nitrogen of leaves from 10 different localities was 2.26 per cent.

the nutrient liquid was kept constant, because calcium carbonate was mixed with the sand, contained after having got ammonia chloride 2.35 per cent of nitrogen in the leaves, and after having got sodium nitrate 2.32 per cent, thus in both cases nearly the normal contents of nitrogen. The plants on the other hand from the sand cultures to which ammonia nitrate had been added and in which the hydrogen ion concentration was not kept constant, why the plants finally died, contained 3.28 per cent of nitrogen in the leaves, thus an abnormally great quantity of nitrogen<sup>1)</sup>.

Of course it is not possible with certainty to state the reason hereof, but perhaps it may be explained in the following manner: When the hydrogen ion concentration of the nutrient liquid on account of the absorption of ammonia of the plant increases to a value that is critical for the plant, a part of the acid of the nutrient liquid, which has been liberated penetrates into the cellular juice of the plant, and consequently the hydrogen ion concentration of the cellular juice increases. To counter-balance this effect, the plant absorbs more ammonia which is used to neutralize the acids. Thus the hydrogen ion concentration of the nutrient liquid further increases, more acid penetrates, and the plant, therefore, must absorb ammonia and so on. In this way a considerable quantity of ammonia is accumulated in the plant.

That the hydrogen ion concentration of the cellular juice really increases when the plants grow under such conditions may be pointed out by aid of litmoid paper. In *Tussilago* the hydrogen ion concentration of the leaf juice under normal conditions has a value of about 6.2<sup>2)</sup>, expressed in  $p_{H^+}$ , but in plants that have developed in sand culture with ammonia nitrate as source of nitrogen as described at the experiments on page 123—130, the  $p_{H^+}$  value of the juice was found to be 4.8 a short time before the plants began to fade. The plants, on the other hand, that had developed with sodium nitrate as source of ni-

1) *Tussilago* plants, on the other hand, which were cultivated in sand that daily was watered with a nutrient solution only containing nitrogen as nitrate and which was so acid, that the growth of the plants was checked, contained 2.30 per cent of nitrogen in the leaves, i. e. the normal quantity.

2) The  $p_{H^+}$  value of the juice in plants from very different localities in Nature was found to vary from 6.1—6.3 (measured both electrometrically and with lacmoid paper).



trogen showed the  $p_{H^+}$  value (about 6.2), which is normal for the cellular juice.

### **The ability of the plants to alter the hydrogen ion concentration of the nutrient liquid.**

It appears from the experiments described above that the plants can alter the hydrogen ion concentration of a nutrient liquid, and that the direction in which the alteration takes place depends on the nature of the source of nitrogen. In order closer to examine the question a number of species were cultivated in water culture during the summer 1919 partly with ammonia chloride and partly with sodium nitrate as source of nitrogen. The plants which were used for these experiments were *Deschampsia flexuosa*, *Senecio silvaticus*, *Tussilago farfara* and *Koeleria pyramidata* as also the culture plants *Secale cereale*, *Triticum sativum*, *Avena sativa*, *Hordeum distichum*, *Lupinus luteus*, *Fagopyrum sagittatum* and *Medicago sativa*. At the experiments solution *a* mentioned on page 132 was used as nutrient liquid containing ammonia chloride, and solution *b* mentioned on the same page as nutrient liquid containing nitrate. A mixture of equal parts of the solution *a* and *b* was used as nutrient liquid containing ammonia nitrate.

The  $p_{H^+}$  values of the solutions were originally 5.6. The cultures were cultivated in duplicate. During the cultivation a constant current of atmospheric air was conducted through the nutrient liquids, so that the carbonic acid liberated at the respiration was driven off<sup>1)</sup>.

<sup>1)</sup> In a single case *Deschampsia flexuosa* was cultivated in a nutrient solution with ammonia chloride as source of nitrogen no airing taking place. The hydrogen ion concentration of the nutrient solution gradually increased, but it increased more in the liquid round the roots of the plants than in the other nutrient liquid. For when the plant was taken out of the liquid, the liquid dropping from the roots appeared to be much more acid than the liquid in the glass. The difference was not equalized after the plants having been placed in the glass again, and this had been shaken some times. Not until the glass was shaken energetically for a long time I succeeded in having the hydrogen ion concentration homogeneous throughout the liquid. Such a case, however, certainly only occurs when cultivating a plant that as *Deschampsia flexuosa* has a bulky bunch of fibrous roots hanging in the middle of the liquid embosoming part of the nutrient liquid. When constant airing of the liquid takes place, the hydrogen ion concentration on the other hand always was homogeneous throughout the liquid.

In the solutions in which ammonia chloride and ammonia nitrate respectively was the source of nitrogen, the hydrogen ion concentration increased as the plants grew until the value critical for each single species was reached, after which the plants died. The critical hydrogen ion concentration occurred in the nutrient liquids containing ammonia chloride at an earlier time than in those containing ammonia nitrate. The highest hydrogen ion concentration produced by the plants in the nutrient liquid was colorimetrically measured and is stated in table 42, in which the  $p_{H^+}$  values are indicated. It will be seen from this table that each single species alters the hydrogen ion concentration of the nutrient liquid to a fixed value and that this value differs for the different species, the acid soil plants altering the hydrogen ion concentration to a higher value than the alkaline soil plants. Rye and oats alter the hydrogen ion concentration to a higher value than barley and wheat, and the conclusion may be drawn that the two first-mentioned cereals can stand a higher hydrogen ion concentration than the two last-mentioned ones.

In solution *b* in which the source of nitrogen was sodium nitrate, the plants altered the hydrogen ion concentration of the nutrient liquid in alkaline direction, by which chlorosis gradually became a fact. However, it was not possible here to determine the lowest hydrogen ion concentration at which the plant can grow, because the death of the plants did not occur as suddenly as was the case with the experiments described above. The lowest hydrogen ion concentration found in the nutrient liquids cannot be considered as the critical limit, as the hydrogen ion concentration of the liquids continued to decrease also after the plants having died; this was due to the incipient decay of the roots, by which ammonia was formed. On the other hand it was observed that chlorosis began when the hydrogen ion concentration of the liquids had reached a fixed value, different for the different species. Thus *Deschampsia flexuosa*, *Senecio silvaticus* and *Lupinus luteus* became chlorotic already when the nutrient liquid had reached a  $p_{H^+}$  value of about 6, while the other species did not become chlorotic until the nutrient liquid had reached a  $p_{H^+}$  value of about 7.2.

In a single case the hydrogen ion concentration of the nutrient liquid fell to a value of 6.2 expressed in  $p_{H^+}$ ; it did not decrease further. The yellow lupine that grew in this nutrient

liquid became slightly chlorotic, but continued to grow though very slowly. In no case, however, it was observed that the plants could make the hydrogen ion concentration of the nutrient liquid increase, when the source of nitrogen was nitrate. Based on these experiments, the plants therefore cannot be ascribed the ability of regulating themselves the hydrogen ion concentration of the nutrient liquid, as Hoagland (1918, 1919) and Arrhenius (1920) hold. At Hoagland's water culture experiment the plants grew in a nutrient liquid containing nitrate, and the alterations of the hydrogen ion concentration of the liquid in most cases therefore passed in alkaline direction. Hoagland, however, states that in a few cases where the hydrogen ion concentration of the nutrient liquid decreased until a  $p_H$  value of 7.4, he observed that the hydrogen ion concentration afterwards increased to a  $p_H$  value of 6.8. I have never made such an observation at my experiments<sup>1</sup>). It cannot be said what was the source of nitrogen at Arrhenius' experiments, as he has not said anything of the manner in which they were carried out. Weis (1919) also thinks that the plants to a certain degree can regulate the hydrogen ion concentration of the nutrient liquid. Weis namely cultivated maize in slightly alkaline reacting nutrient liquids. In these liquids the plants were thriving badly until a certain time at which the growth suddenly began to increase. At the same time Weis observed "an interesting shifting in the hydrogen ion concentration", as the  $p_H$  value of the nutrient liquids was altered from 7.35 to 5.86. "The solution", Weis says, "was here such that the alteration of the reaction might take place according to the needs of the plants as the source of nitrogen was ammonia nitrate (l. c. p. 268)". Evidently Weis considers ammonia nitrate as physiologically neutral, but as will be seen from my experiments this is not correct. The ammonia nitrate is physiologically acid, and the alteration of the hydrogen ion concentration observed by Weis therefore only could take place in acid direction and would have

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<sup>1</sup>) It must be remembered that the greater part of the carbonic acid at my experiments was driven off the nutrient liquids by means of atmospheric air. If the carbonic acid had not been driven off, the hydrogen ion concentration in the nutrient liquids containing nitrate would have been higher than it was found to be. It is an open question whether the plants can regulate the disengagement of carbonic acid and thus act regulating on the hydrogen ion concentration of the nutrient liquid, but it is not very probable.

Table 42. The hydrogen ion concentration of the nutrient solutions expressed in  $p_{H^+}$  at the time when the plants were dying.

	Source of nitrogen	
	$NH_4 Cl$	$NH_4 NO_3$
	$p_{H^+}$	$p_{H^+}$
<i>Deschampsia flexuosa</i> (experiment 1) .....	2.2	2.3
— — — 2 .....	2.2	2.2
<i>Senecio silvaticus</i> (experiment 1) .....	2.5	2.6
— — — 2 .....	2.6	2.5
<i>Tussilago farfara</i> (experiment 1) .....	3.3	3.4
— — — 2) .....	3.4	3.4
<i>Koeleria pyramidata</i> (experiment 1) .....	3.5	3.5
— — — 2) .....	3.5	3.5
<i>Secale cereale</i> (experiment 1) .....	2.6	2.7
— — — 2 .....	2.6	2.6
<i>Avena sativa</i> (experiment 1) .....	2.7	2.8
— — — 2) .....	2.8	2.7
<i>Lupinus luteus</i> (experiment 1) .....	2.8	2.8
— — — 2 .....	2.9	2.8
<i>Fagopyrum sagittatum</i> (experiment 1) .....	3.0	3.1
— — — 2) .....	3.1	3.1
<i>Triticum sativum</i> (experiment 1) .....	3.2	3.2
— — — 2 .....	3.2	3.2
<i>Hordeum distichum</i> (experiment 1) .....	3.4	3.4
— — — 2 .....	3.3	3.3
<i>Medicago sativa</i> (experiment 1) .....	3.4	3.4
— — — 2 .....	3.3	3.4

taken place in this direction, also if the nutrient liquid originally had had a  $p_{H^+}$  value of e. g. 4.5; in this case the hydrogen ion concentration would quickly have involved the death of the plants.

It will appear from the above described experiments made by me that of the physiologically acid solution *a* and the physiologically alkaline solution *b*, a physiologically neutral solution

can be produced by mixing the two solutions in suitable proportion. It was found that a mixture of three volumes of solution *a* and seven volumes of solution *b* was nearly physiologically neutral, for when the plants were cultivated in this solution only insignificant alterations of the hydrogen ion concentration took place, also when the hydrogen ion concentration had a  $p_{H}$ -value unfavourable to the plants<sup>1)</sup>.

Thus it will be seen from the experiments that the direction of the alterations produced by the plants in the nutrient liquids is determined by the nature of the source of nitrogen.

As mentioned above (p. 135), *Tussilago* plants cultivated in sand culture with ammonia nitrate as source of nitrogen contained an abnormally great quantity of nitrogen when the hydrogen ion concentration critical for the plants occurred in the nutrient liquid. It was also the case at the experiments described here, as *Tussilago* plants from the nutrient liquids containing ammonia nitrate contained 3.52 per cent of nitrogen, the plants from the nutrient liquids containing ammonia chloride 3.58 per cent, while the plants from those containing sodium nitrate contained 2.41 per cent.

Plants of the yellow lupine and buckwheat taken from the same experiment were also analyzed. Plants of yellow lupine from the nutrient liquids containing ammonia chloride appeared to contain 4.92 per cent of nitrogen, while plants of the same species from those containing sodium nitrate contained 3.36 per cent; buckwheat plants from the liquids containing ammonia chloride contained 5.63 per cent of nitrogen and the plants from those containing sodium nitrate 1.93 per cent.

Finally it appeared from the same experiments — as was also the case with the experiments described on p. 136 — that plants cultivated in the nutrient liquids containing ammonia chloride and ammonia nitrate when the critical hydrogen ion concentration occurred in the nutrient liquid, showed a hydrogen ion concentration of the cellular juice that was higher than normal for the plants.

That the hydrogen ion concentration of the cellular juice may be altered in the direction of the hydrogen ion concentra-

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<sup>1)</sup> At the experiments described at p. 113 the nutrient liquid therefore had just the same composition as the said mixture of solution *a* and *b*.

tion<sup>1</sup> of the nutrient liquid, also when the plants grow in solutions the hydrogen ion concentration of which is considerably lower than that at which the plant reached its most vigorous growth, was shown with regard to *Senecio silvaticus*. The highly chlorotic individuals of this species growing in solution *b*, the  $p_{H^+}$  value of which at the time when the plants were examined was 6.5, showed a  $p_{H^+}$  value of 6.2 for the leaves and the stalks, while the cellular juice of normal individuals of *Senecio silvaticus* from Grib Forest had a  $p_{H^+}$  value of 5.5.

### Are aluminium ions toxic to alkaline soil plants?

Hartwell and Pember hold — as mentioned on p. 12 — that they have shown that aluminium ions have a toxic effect on barley but not on rye. This observation has made the two investigators see the "lime-question" in an essentially different light than was hitherto the case, as they consider lime-loving plants (alkaline soil plants), under which barley must be subsumed, as plants to which aluminium ions are toxic, and which consequently cannot thrive in very acid soils, in which always are found small quantities of aluminum in solution, while the species which as rye are thriving comparatively well in very acid soils are not affected by aluminium ions.

That aluminum salts in solution even in small quantities have a restrictive influence on the growth of the different species in water culture as also in sand culture has furthermore been shown by Hébert (1907), House and Gies (1905), Kratzmann (1914), Ruprecht (1915), Stoklasa (1918 II) and Mirasol (1920)<sup>2</sup>.

<sup>1</sup>) Haas (1920) states that the hydrogen ion concentration of the cellular juice decreases (about 0.1—0.3 in  $p_{H^+}$ ) when calcium carbonate is added to the soil in which the plants grow. Clevenger (1919) has made the same observations as regards the root juice, but he states that with regard to the overground parts of the plants he had observed an increase of the hydrogen ion concentration of the cellular juice. Hoagland (1919 II) was unable to note on barley plants cultivated in nutrient liquids of different hydrogen ion concentrations, that the hydrogen ion concentration of the cellular juice was affected by that of the growing medium.

<sup>2</sup>) Stoklasa (1918 I) has examined the occurrence of aluminum in a number of plants and found, that hygrophyte plants contained much aluminum especially in the roots. Thus aluminum forms 3 per cent of the dry substance in the roots of *Scirpus maritimus*; also *Rumex maritimus* and many cyperaceae contain ample quantities hereof; mesophytes contain less, *Tussilago farfara*

It must, however, be remembered that aluminum salts are very hydrolized, consequently they give a very acid reaction. Thus addition of aluminum salts to nutrient liquids will cause the hydrogen ion concentration to increase, and the toxic effect may therefore be due to a too high hydrogen ion concentration in the nutrient liquid. Miyake thought this possible, and therefore examined the question more closely. He found that the toxic effect of aluminum chloride on rice plants in water culture manifested itself when the concentration was greater than  $n/_{7500}$ . A solution of hydrochloric acid of corresponding normality had a corresponding toxic effect, but as the latter had a much higher hydrogen ion concentration than the corresponding aluminum chloride solution, Miyake draw the conclusion that it is not the hydrogen ions originating with the hydrolosis of the aluminum salt, that has the toxic effect on the plants but the aluminum ions themselves.

The experiments hitherto made on the toxicity of the aluminum, however, are not satisfactory, and it cannot be said to be proved that aluminum ions exercise toxic effects on the plants, for i. a. the hydrogen ion concentration of the nutrient liquids and the possible alterations produced by the plants during the growth have not been controlled. It is also admitted by Hartwell and Pember that the experiments must be made at constant hydrogen ion concentration before any certain conclusion can be drawn. Furthermore it must be remembered that aluminum ions more or less completely precipitate the phosphoric acid in the nutrient liquid, consequently the plants may be in want of phosphoric acid, in which case the growth of the plants of course is checked, but this obviously is not due to the aluminum ions. In order to avoid precipitation hereof, Hartwell, and Pember at their experiments have used a very small quantity of phosphate, viz. only about 10 mg  $\text{KH}_2\text{PO}_4$  in one litre of nutrient liquid. If an essential part of the quantity of phos-

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thus 0.5 per cent, while xerophytes are poorest on this substance, as generally they only contain about 0.01 per cent of aluminum. Water culture experiments made by Stoklasa (1918 II) in conformity herewith gave the result that xerophytes were very susceptible to aluminum compounds, while the hygrophile plants were not checked in their growth until far higher hydrogen ion concentrations were reached. Very small quantities of aluminum even had a stimulating effect on the growth of these plants.

phate which beforehand is very small is precipitated by aluminum ions, the plants will suffer from want of phosphoric acid, especially — as was the case at the experiments of the two above mentioned investigators — as 6 barley plants were cultivated in only 250 cc of nutrient liquid.

In order to try the theory set up by Hartwell and Pember I made a number of experiments with *Secale cereale* and *Hordeum distichum* as also with *Senecio silvaticus* and the alkaline soil plants *Tussilago farfurus* and *Koeleria pyramidata*. According to the theory of the said two investigators, aluminum ions are said to be especially toxic to the two latter species.

Previous to the final experiments, a number of experiments were made with rye and barley (Tystofte Chevalier barley); for these experiments a nutrient liquid was made which in one litre boiled and filtered tap water contained:

1.12	g	NaNO <sub>3</sub>
0.30	-	NH <sub>4</sub> Cl
0.25	-	Ca SO <sub>4</sub> , 2H <sub>2</sub> O
0.12	-	Mg SO <sub>4</sub> , 7H <sub>2</sub> O
0.20	-	K Cl
0.01	-	KH <sub>2</sub> PO <sub>4</sub>
0.003	-	Fe Cl <sub>3</sub>

This nutrient liquid is almost physiologically neutral and contains only a small quantity of phosphoric acid as was also the case with that used by Hartwell and Pember. The two cereals were cultivated in this nutrient liquid at three different hydrogen ion concentrations ( $p_H$  3.5, 4.5 and 6.0) partly with, partly without aluminum, 10 cm<sup>3</sup> 0.1 n of dissolved alun<sup>1)</sup> in the experiments in which the plants were cultivated with aluminum were added to 1 litre of the nutrient liquid, which gives 9 mg aluminum on each litre<sup>2)</sup>. It appeared that both rye and barley at all the three said hydrogen ion concentrations only showed a poor growth, when aluminum was found in the nutrient liquid, while they were thriving normally when this was not the case<sup>3)</sup>.

<sup>1)</sup> This solution contained 15.8 g alun per litre.

<sup>2)</sup> The quantity of aluminum used is somewhat greater than that used at Hartwell and Pember's experiments. At some of my experiments larger quantities (upto 10 times as much) were added, but this did not affect the development of the plants otherwise than the smaller quantities.

<sup>3)</sup> In the solutions the  $p_H$  value of which was 3.5 the barley would not deve-



In the solutions containing aluminum and the  $p_{H^+}$  value of which was 4.5 rye and barley, however, behaved somewhat differently, as the barley after being placed in the aluminous nutrient liquid did not develop new adventitious roots, while rye developed several new roots, but besides, as mentioned above, the growth as regards both species was poorer than in the non-aluminous nutrient liquid of the same hydrogen ion concentration. That the plants showed a poorer growth in the aluminous nutrient liquids than in the non-aluminous ones may, however, be due to want of phosphoric acid, for although a very small quantity of phosphoric acid<sup>1)</sup> was found in the above described nutrient liquid, precipitate<sup>2)</sup> appeared when the alun-solution was added, which appeared to consist of phosphate of aluminum. The quantity of the deposit depended on the hydrogen ion concentration of the nutrient liquid, the precipitate being smallest in the most acid nutrient liquid ( $p_{H^+}$  3.5), greatest in the least acid one ( $p_{H^+}$  6.0). In order to examine how great a part of the phosphoric acid was precipitated of the said quantity of alun (10 cm<sup>3</sup> 0.1 n alun solution on each litre), the three solutions the  $p_{H^+}$  values of which after having added alun were 3.5, 4.5 and 6.0 respectively, were filtered and the quantity of phosphoric acid was determined in the filtrate according to the method of Neumann and Gregersen (Gregersen 1907). In the nutrient liquid the  $p_{H^+}$  value of which was 3.5, 2.47 mg PO<sub>4</sub> on each litre was found in the filtrate corresponding to 3.53 mg KH<sub>2</sub> PO<sub>4</sub> (10 mg added); in the solution the  $p_{H^+}$  value of which was 4.5, 0.38 mg PO<sub>4</sub> was found corresponding to 0.54 mg KH<sub>2</sub> PO<sub>4</sub>; in the latter nutrient liquid thus only about 1/20<sup>th</sup> of the original quantity of phosphoric acid solution that had been added was found. In the solution the  $p_{H^+}$

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lop at all, because this species, as will be seen from the above mentioned experiments, cannot thrive in solutions of so high hydrogen concentration.

1) At a number of experiments made in 1918 with the same two species a nutrient liquid containing a great quantity of phosphoric acid was used; at these experiments no difference in the growth of the plants appeared, whether the nutrient liquid contained dissolved alun or not, and the reason hereof obviously was that the aluminum was precipitated by the phosphoric acid.

2) This is also the case with the nutrient liquid used by Hartwell and Pember which I have reproduced. The precipitate in this nutrient liquid is — it is true — not directly visible, as on account of the insignificant salt-concentration of the solution it does not flock out. But if a little sodium chloride is added, the deposit is immediately flocking out.

value of which was 6.0, the quantity of phosphoric acid was so small as not to allow a determination in one litre of the filtrate.

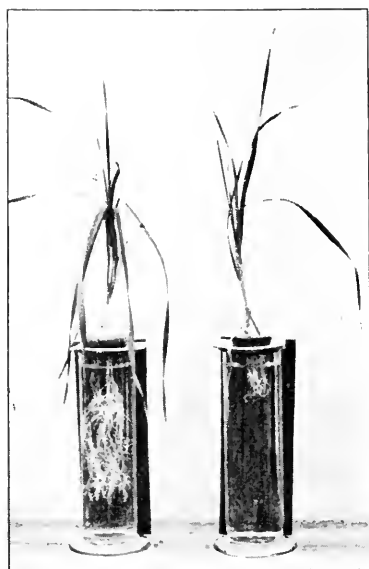
In the said provisional experiment the quantity of phosphoric acid thus was not the same in the aluminous as in the non-aluminous nutrient liquids, and the poorer development of the plants that has been noted in the aluminous liquids therefore may as well be supposed to be due to want of phosphoric acid as to the presence of the aluminum ions.

At the final experiments the nutrient liquids therefore were composed in such a way that the same quantity of phosphoric acid in solution was found in both the aluminous nutrient liquids as in the non-aluminous ones. Nutrient liquids were used, the  $\text{pH}$  values of which were 3.5 and 4.5 respectively, and the aluminous liquids were made in quite the same manner as for the provisional experiment, viz. by adding 10  $\text{cm}^3$  0.1 N dissolved alun to one litre of the nutrient liquid that was composed as that described on p. 144; by means of hydrochloric acid or sodium hydroxyde the solution was then given the desired hydrogen ion concentration. The non-aluminous nutrient liquid also had the same composition as that described on p. 144 except of course as regards the quantity of phosphoric acid, as the nutrient

**Table 43.** The weight (g) of the plants at the end of the experiment.

	$\text{pH} \cdot 3.5$		$\text{pH} \cdot 4.5$	
	without Al.	with Al.	without Al.	with Al.
<i>Senecio silvaticus</i> (1 plant), fresh ..	10.00	10.50	14.40	14.30
— — (1 — ), dry....	1.50	1.53	2.00	2.15
<i>Secale cereale</i> (1 plant), fresh .....	2.80	2.90	4.00	3.80
— — (1 — ), dry.....	0.31	0.33	0.48	0.47
<i>Hordeum distichum</i> (1 plant), fresh.	»	»	5.30	3.15
— — (1 — ), dry ..	»	»	0.71	0.42
<i>Tussilago farfara</i> (1 plant), fresh ..	»	»	4.23	4.40
— — (1 — ), dry....	»	»	0.52	0.55
<i>Koeleria pyramidata</i> (5 plants), fresh.	»	»	3.05	3.12
— — (5 — ), dry..	»	»	0.39	0.40

liquid, the  $p_{H^+}$  value of which was 3.5, contained 3.53 mg and the nutrient liquid the  $p_{H^+}$  value of which was 4.5, 0.54 mg primary potassium phosphate on one litre (the quantities found at the analysis described on p. 145). The quantity of phosphoric



Without Al.      With Al.

Fig. 27. *Hordeum distichum* in water culture without and with aluminum respectively. Note the defective development of the root in the aluminous nutrient liquid.

acid in these nutrient liquids thus was the same as that found in solution in the corresponding aluminous nutrient liquids. As only very small quantities of phosphoric acid were found in all the nutrient liquids, these were changed during the period of the experiment to prevent the plants from suffering from want of phosphoric acid. At the beginning they were changed every second day, later when the plants began to grow quicker, every day. By thus frequently changing the nutrient liquid, the plants did not get opportunity to alter the hydrogen ion concentration.

*Senecio silvaticus* and rye were cultivated both in nutrient liquids the  $p_{H^+}$  values of which were 3.5 and 4.5 respectively, the other species on the other hand only in nutrient liquids the  $p_{H^+}$  value of which was 4.5, as these species cannot develop at much higher hydrogen ion concentrations.

The experiments were commenced on the 18th of June and lasted to the 30th of July. The weight of the plants at the end of the experiment will appear from table 43. It will be seen from this table that barley plants are highly checked in their growth, if the nutrient liquid contains aluminum ions and  $p_H$  at the same time has a value of 4.5. In the aluminous nutrient liquid the barley plants did not develop any new adventitious roots, a fact that will be seen from fig. 27. Thus aluminum ions have a restrictive influence on the development of the roots of barley; therefore it must be laid down that aluminum ions really have a toxic effect on barley. Regarding the other species no checking of the development of the root was observed in the nutrient liquids containing aluminum, and it will be seen from the table that the plants reached the same weight, whether they grew in aluminous or non-aluminous nutrient liquids. According to the theory of Hartwell and Pember, aluminum ions are supposed to be especially toxic to alkaline soil plants, but as will appear from the experiment, aluminum ions have no toxic effect on the alkaline soil plants *Tussilago farfarnus* and *Koeleria pyramidata*. Therefore, it does not in general hold good that alkaline soil plants cannot thrive in very acid soils, because small quantities of dissolved aluminum compounds are found in such soils.

## CLOSING REMARKS.

As shown in section A, the composition of the vegetation varies under natural conditions regularly with the hydrogen ion concentration of the soil. In conformity herewith the experiments described in section B have shown that the plants found in Nature especially in acid soils, the acid soil plants, reach their best growth in nutrient liquids that are considerably more acid than the liquids in which the plant especially found in Nature in slightly acid, neutral and alkaline soil, the alkaline soil plants, reach their best growth. Furthermore the experiments have shown, that alkaline soil plants cannot develop in nutrient liquids of as high hydrogen ion concentration as the acid soil plants, while the latter in the slightly acid nutrient liquids, in which the alkaline soil plants reach their best growth, become chlorotic and only reach a poor growth.

As to the significance for the plant distribution, that possibly might be attributed to two of the factors that vary more or less regularly with the hydrogen ion concentration, viz, 1) the contents of the soil of dissolved aluminum compounds and 2) the course of the transformation processes of the nitrogen the conclusion may be drawn from the culture experiments described in section B, that it is not the contents of dissolved aluminum compounds in the very acid soils, nor their weak or wanting nitrification, that is the reason of the alkaline soil plants not thriving in these soils, for aluminum ions are not toxic to alkaline soil plants in general, and ammonia salts and nitrates have the same value as source of nitrogen both for alkaline soil plants as for acid soil plants<sup>1)</sup>.

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<sup>1)</sup> On the other hand the physiologically acid reaction of the ammonia salts can certainly assert itself in the very acid non-nitrifying but ammonia forming soils (raw humus soils), as the hydrogen ion concentration of the soil liquid in the immediate vicinity of the plant roots increases, when the plants absorb the ammonia. In the raw humus soils the hydrogen ion concentration of the soil liquid in the immediate vicinity of the plant roots therefore without doubt can be considerably higher than in the other parts of the soil. The culture experiments have also shown that a raw humus soil plant as *Deschampsia flexuosa* can thrive in nutrient liquids of a considerably higher

The investigations and experiments may therefore make it highly probable that it is the hydrogen ion concentration of the soil as such that has an essential influence on the composition of the plant formations.

### Resumé.

The hydrogen ion concentration in natural Danish soils varies from 3.4—8.0 expressed in  $p_H$ .

The hydrogen ion concentration of the soil has appeared to have an essential influence on the composition of the plant formations, as the single species only are found in soils, the hydrogen ion concentration of which lies within a certain range characteristic for each single species. Within this range is a narrower range, in which the species has its greatest average frequency.

When the formations are not too poor on species, we can conclude from the composition of the vegetation to the hydrogen ion concentration of the soil.

The number of the species and their density are on an average greatest in soils, the hydrogen ion concentration of which is near the neutral point; by the increasing hydrogen ion concentration of the soil both the number of the species as their density generally decrease.

At a number of water culture experiments it appeared that species, which in Nature only are found on very acid soil (acid soil plants) reached their best growth in nutrient solutions, the  $p_H$  values of which were near 4, while species that in Nature only are found on slightly acid, neutral and alkaline soil (alkaline soil plants) reached their most vigorous growth in nutrient liquids the  $p_H$  values of which were lying between 6 and 7. In the slightly acid nutrient solutions, in which alkaline soil plants reached their best growth, the acid soil plants did not thrive well and became chlorotic. A graphic reproduction of the conditions of growth will be seen in fig. 14, p. 116.

The theory set up by Hartwell and Pember, according to which the wanting ability of the alkaline soil plants to thrive

hydrogen ion concentration than the soil liquid, which by means of a hydraulic press may be pressed out of a raw humus soil, and which in general has a  $p_H$  value of about 3.5.

in very acid soils, is not due to these plants not being able to stand as high hydrogen ion concentrations as the acid soil plants, but on the other hand is due to small quantities of dissolved aluminum compounds being found in the very acid soils, which are said to be toxic to alkaline soil plants but not to acid soil plants, has shown not to be of general validity, as experiments have proved that aluminum ions are not toxic to alkaline soil plants in general.

The theory advanced by Bear and other investigators that acid soil plants are plants that are able to use ammonia nitrogen while alkaline soil plants demand nitrate nitrogen, and therefore cannot thrive in very acid soils, in which the nitrification is poor or wanting, is not correct, as experiments have proved that ammonia nitrogen and nitrate nitrogen are of the same value as source of nitrogen both for acid soil plants and alkaline soil plants.

It furthermore appears from the investigations, that the quantity of plant nutrients of the soil which by some investigators are considered partly to depend on the hydrogen ion concentration of the soil, as the acid soils are considered to be poor on plant nutrients, the neutral, and alkaline soils to be rich on these nutrients, does not to any greater extent influence the plant distribution. It also appeared that there are alkaline soils which are poor on nutrients, and their vegetation shows no resemblance to the vegetation of the very acid soils which are poor on nutrients.

Therefore it is probable that it is the hydrogen ion concentration of the soil as such which exercises a considerable influence on the composition of the vegetation.

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The soil analysis and culture experiments described in this treatise are carried out in the Chemical Department of the Carlsberg Laboratory, where I have worked as a guest, until on the 1st of June 1919 I was appointed assistant there. I convey my best thanks to Professor S. P. L. Sørensen, D. Sc. and Director of the Laboratory for the readiness with which I was given access to the Laboratory to work there with my experiments. I am also very beholden to him and Professor C. Raunkiær, my university professor at botany for the interest, with which they

have watched my work and at any time been prepared to advise and direct me.

I also thank Professor Eug. Warming, D. Sc. both for the interest he has taken in my investigations and because through his kindness I was allowed to use the green house in the garden of Carlsberg for my culture experiments. I am also indebted to Mr. Wagenblast, the gardener, because year after year he afforded room for my experiments.

I further owe thanks to the Board of Directors of the Botanic Travelling Fund, who have granted me money, to Mr. B. Boysen Jensen, D. Sc. who has given me good advice as to the practical arrangement of the culture experiments, and to Mr. Bornebusch, graduate in forestry, who on my direction has made the formation-statistical analysis on some of the localities mentioned in this treatise in Store Bogeskov, as also he has forwarded soil samples from the said localities to me.

*April 1921.*



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COMPTES-RENDUS

DES TRAVAUX

DU

# LABORATOIRE CARLSBERG

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15<sup>ME</sup> VOLUME.

No. 2



COPENHAGUE

EN COMMISSION CHEZ H. HAGERUP

IMPRIMERIE DE THIELE

1923

*Prix: 75 Øre*

LES COMPTES-RENDUS  
DES TRAVAUX DU LABORATOIRE CARLSBERG

paraissent par livraisons à des époques indéterminées. A mesure qu'il en paraîtra un nombre suffisant pour faire un volume, les abonnés recevront un titre en même temps qu'une table des matières, avec l'indication de la période qu'embrasse le volume.



# STUDIES ON PROTEINS.

BY

S. P. L. SØRENSEN.

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## VI. ON CRYSTALLINE EGG-ALBUMIN SALTS, PRECIPITATED BY SALTS OTHER THAN SULPHATE OF AMMONIA.

BY

S. P. L. SØRENSEN AND S. PALITZSCH.

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In a previous work<sup>1)</sup>, the question as to composition of the egg-albumin crystals precipitated by sulphate of ammonia was subjected to thorough discussion on the basis of extensive experimental material. The main result of this may be briefly expressed as follows: The egg-albumin crystals consist for the most part of egg-albumin and water, but probably always contain, in addition, a certain very small amount of sulphuric acid, and should be regarded as composed of hydrous egg-albumin sulphate. We need not here go into the question as to how the amount of surplus sulphuric acid in the crystals may be presumed to vary with the concentration of hydrogen ions, but will deal exclusively with one of those features which, as stated in the mentioned paper<sup>2)</sup>, "will be most easily understood if we suppose the crystallised precipitate to contain a slight dose of sulphuric acid."

We are here referring to the fact that at the time when that paper was written, neither F. Gowland Hopkins nor we ourselves had succeeded, despite numerous experiments with many different salts, in crystallising egg-albumin by means of other substances than sulphates.

Like E. G. Willcock<sup>3)</sup> we had found sulphate of magnesia applicable for the crystallisation of egg-albumin. More particularly

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<sup>1)</sup> S. P. L. Sørensen & Margrethe Høyrup: *Comptes-rendus du Laboratoire Carlsberg* **12**, 164 (1917), *Zeitschr. physiol. Chem.* **103**, 211 (1918).

<sup>2)</sup> *I. c.* **12** p. 199 and **103** p. 251.

<sup>3)</sup> *Journ. of Physiol.* **37**, 32 (1908).

however, we had on several occasions obtained good results with a saturated solution of the sulphates both of sodium and potassium, which proved an excellent precipitant in crystallising egg-albumin.

While the paper was in the press, however, we succeeded, as mentioned in a postscript<sup>1)</sup> to the paper, in precipitating egg-albumin in a crystalline form by the use of a suitable mixture of primary and secondary phosphate of ammonia.

Since then, one of us, S. Palitzsch, using an egg-albumin solution thoroughly purified by ammonium sulphate and thereafter cleansed of the latter by dialysis, succeeded in getting out the egg-albumin in a crystalline state. The precipitant here employed was a suitable mixture, either of primary and secondary ammonium arsenate, or of primary and secondary ammonium citrate.

By analogy with the view we have previously expressed regarding the crystalline precipitate obtained when using ammonium sulphate, we must presume that we have here crystalline salts of egg-albumin with phosphoric, arsenic and citric acid respectively. Under the microscope, however, no difference is discernible between these various egg-albumin salts, all of which appear, like the sulphate, as small or somewhat larger, needle-shaped crystals, or at times, especially with slow crystallisation, in the form of long, well-developed prismatic crystals. Direct microscopical examination thus affords no means of determining whether we have here to deal with several different salts, or whether in all four instances it is merely a case of crystallising out the hydrous egg-albumin. We have therefore endeavoured to arrive at an answer of the question by another route, viz. by determination of the factor  $r$  in the crystalline phosphate.

As set forth in detail in the mentioned paper, we understand by  $r$  the factor by which the weight of protein-nitrogen must be multiplied in order to give the weight of hydrous egg-albumin. The factor  $r$  is the only constant hitherto determined for crystallised egg-albumin sulphate, and its value is 7.86, while the factor by which the weight of protein-nitrogen must be multiplied to give the weight of water-free egg-albumin is only 6.4. It is thus easy to calculate that crystallised egg-albumin

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<sup>1)</sup> l. c. **12** p. 212 and **103**, 266.

sulphate contains abt. 0.22 gr. of water per gr. water-free egg-albumin.

It might now be imagined that the precipitate crystallised out by means of ammonium phosphate showed a different water-content from that obtained with ammonium sulphate, which would be apparent in the fact that  $r$  was of another value than 7.86. Should this prove to be the case, we should then have definite proof that the two cases did not represent a crystallisation of the same hydrous egg-albumin, but in all probability a crystallisation of two different salts. On the other hand, it must be pointed out that the opposite conclusion would not be justified, since, in the first place, it is not impossible that two distinct salts of the same base might crystallise with the same water-content, and further, it is natural to suppose that the hydrous egg-albumin, which in both cases makes up by far the greater part of the crystallised deposit, is probably also the sole determining factor in regard to both shape and water-content of the crystals.

From the results of the work described in the experimental section, it now appears that the factor  $r$  was found to be different in the two series of experiments, in which the crystallisation of egg-albumin phosphate took place at varying concentrations of hydrogen ions and varying concentration of ammonium phosphate, which latter, in order to give a good and abundant, but also fine and therefore not too rapid crystallisation, had to be adapted to the concentration of hydrogen ions.

In the one series of experiments, the  $p_H$  of the mother liquor was found equal to 4.81, and the content of ammonia-nitrogen in 100 gr. mother liquor 3.2994 gr.; in this series,  $r$  appeared as equal to 7.89.

In the other series of experiments, the  $p_H$  of the mother liquor was found equal to 5.48 and the content of ammonia-nitrogen per 100 gr. mother liquor 4.4572 gr.; in this series,  $r$  was found equal to 8.12.

These experimental series can hardly be said to provide any decisive proof that the deposit obtained by means of ammonium phosphate differs from that obtained with ammonium sulphate. The first series, made at a concentration of hydrogen ions answering to the isoelectric reaction of the egg-albumin ( $p_H = 4.8$ ) gave  $r$  as = 7.89, i. e. within the limits of error to

precisely the same value as that found for egg-albumin sulphate.

The second series of experiments was carried out at a concentration of hydrogen ions considerably lower than the isoelectric reaction of the egg-albumin, and it might therefore be thought that the crystallised precipitate would have bound large quantities of ammonia; this would, however, as will be seen from the formula whence  $r$  is calculated, give too low a value of  $r$ , so that this explanation of the high value arrived at ( $r = 8.12$ ) must doubtless be rejected. Nor, again, is it justifiable, in our opinion, to explain the difference between  $r = 8.12$  and  $r = 7.86$ , no inconsiderable one in itself, as merely an experimental error, still less as the third series of experiments described in the experimental section affords no proof that 8.12 is too high a value for  $r$  at low concentrations of hydrogen ions.

We therefore consider that the result of this second series of experiments must rather be said to suggest that we have here another egg-albumin salt than that precipitated by ammonium sulphate, though, as already mentioned, the proof is not, in our opinion, decisive.

Finally, we should merely remark that the work with ammonium phosphate as a precipitant, while not perhaps presenting any essential difficulty, is yet by no means so easy as with ammonium sulphate, partly from the fact that the solubility of the secondary, and especially of the primary ammonium phosphate in water is far inferior to that of ammonium sulphate; and further, because alterations in the salt concentration also give essential differences in the concentration of hydrogen ions, unless the ratio between primary and secondary salt be simultaneously adjusted.

The investigations here dealt with were carried out during the years 1917—18, and as we have not since then had further opportunity of continuing these studies, we thought it best to publish the experimental material obtained to date. For the rest, we have, from what we have learned in the course of these investigations, no doubt but that the solubility and other qualities of egg-albumin phosphate are determinable in precisely the same manner and according to the same principles as previously applied in our studies of egg-albumin sulphate.

## Experimental Section.

### a. Preparation of egg-albumin phosphate.

In the preparation of egg-albumin phosphate, the precipitants employed were: —

- 1) solid primary ammonium phosphate,
- 2) a saturated solution of the same, and
- 3) a solution of secondary ammonium phosphate,

this last prepared by dissolving 1 gr. mol. (115 gr.) of primary ammonium phosphate in 100 ccm. 10 n. ammonia water together with sufficient water to dissolve the whole; the volume of this solution amounted to 290 ccm., so that 100 ccm. of solution would contain abt. 40 gr. of the primary salt, and abt. 35 ccm. 10 n. ammonia water (the solution is termed "secondary").

By means of these precipitants and a few other, similar mixtures designed for special purposes, it is possible to vary the concentration of hydrogen ions and the concentration of salt, and thus also the precipitating conditions within a fairly wide margin.

The primary ammonium phosphate was prepared from pure phosphoric acid by dissolving in water, adding the calculated quantity of ammonia with proper cooling and recrystallisation of the crystallised salt. The mother liquor was used as mentioned below.

The best raw material for preparing egg-albumin phosphate is egg-albumin thoroughly purified by repeated recrystallisation with ammonium sulphate.

The ammonium sulphate can then be removed by dialysis, which method we employed in our first experiments, or, more easily where there is a fair abundance of material available, by washing out the crystals precipitated by the ammonium sulphate with a solution of primary and secondary ammonium phosphate in suitable proportions.

Having obtained a dialysed egg-albumin solution, the process is then as follows: To each 100 ccm. egg-albumin solution is added 35 gr. solid primary phosphate and 12 ccm. "secondary". By thoroughly stirring and heating to ordinary temperature, a clear solution is obtained; any denaturated egg-albumin which may be present is to be filtered off. When left to stand, with repeated stirring, the egg-albumin phosphate will crystallise out, most readily, of course, after inoculation, but as a rule quite

readily without. The crystals showed under the microscope the customary appearance, as needles of greater or smaller size, sometimes collected in bundles, sometimes mingled with long, finely developed prismatic crystals. The precipitate is filtered off and washed with a "washing liquid" consisting of 35 gr. solid primary phosphate dissolved in 100 ccm. of water together with 12 ccm. "secondary".

Recrystallisation is carried out simply by dissolving the precipitate with accompanying mother liquor in a measured quantity of water; then the solution — after being filtered if required — is made to crystallise by adding solid primary phosphate and "secondary", the total amount not to exceed 35 gr. solid primary phosphate and 12 ccm. "secondary" per 100 ccm. water used for dissolving the precipitate. The addition may be made all at once, but the crystals come out better when a little is added at a time, and especially when the last portions of primary phosphate and "secondary" are kept back until a considerable amount of the albumin has crystallised.

With the process here described, the crystallisation takes place at a concentration of hydrogen ions approaching the isoelectric reaction of egg-albumin ( $p_H = 4.8$ ), but the concentration of hydrogen ions at which the crystallisation takes place may easily be varied by altering the proportion between solid primary phosphate and "secondary".

Where any considerable quantities are used, it is best to take the purified and crystallised egg-albumin sulphate to start with, this being cleansed of an essential part of the ammonium sulphate by rinsing in a suitable mixture of primary and secondary ammonium phosphate. For this purpose, the mother liquor obtained by recrystallisation of the primary ammonium sulphate can be used, with the addition of a quantity of concentrated ammonia water sufficient to give the undiluted solution a concentration of hydrogen ions answering to  $p_H = 4.7-4.8$ .

The egg-albumin sulphate is taken from the filter and rubbed round in a big dish with five times its volume of the washing liquid, the deposit being then filtered off and washed once or twice with washing liquid. When this operation is again repeated, the deposit contains only a little ammonium sulphate, which can be removed by one or two recrystallisations carried out exactly as above described.

b. Determination of the factor  $r$ .

The determination of the factor  $r$  was carried out in exactly the same manner as in the case of the egg-albumin sulphate,  $r$  being calculated from the formula

$$r = \frac{100 (a_f \div a_b)}{a_f \cdot p_b \div a_b \cdot p_f}$$

where  $a_f$ ,  $a_b$ ,  $p_f$  and  $p_b$  are of the same significance as in our previous experiments<sup>1)</sup>.

Two series of experiments were made, both of ordinary temperature (18°—19°). In No 1, 375 ccm. contained in all abt. 7.5 gr. protein-nitrogen, in No 2, 600 ccm. contained abt. 7 gr. protein-nitrogen; in both cases, the precipitant consisted of a mixture of primary and secondary ammonium phosphate, but the proportion between these two salts was not precisely known. The content of both ammonia and phosphoric acid was somewhat higher in No 2 than No 1, and it could be calculated that each 100 gr. (water plus phosphoric acid) in No 1 contained abt. 22 gr. phosphoric acid and in No 2 24—25 gr. phosphoric acid.

The results of the experiments are shown in Table 1 and need no further explanation.

**Table 1.**  
The factor  $r$ .

Experimental series	Period of crystallisation (Days of 24 hours)	100 gr. of filtrate contained		Concentration of hydrogen ions in the undiluted filtrate $p_H$	100 gr. precipitate with adherent mother liquor contained			$r$	Mean value of $r$
		Ammonia N in gr. ( $a_f$ )	Protein N in gr. ( $p_f$ )		Sample marked	Ammonia N in gr. ( $a_b$ )	Protein N in gr. ( $p_b$ )		
1	11	3.2994	0.7869	4.81	I	2.4821	3.7354	7.880	7.89
					II	2.4842	3.7208	7.898	
					III	2.4427	3.8740	7.889	
2	5	4.4572	0.1055	5.48	I	3.4677	2.8128	8.130	8.12
					II	3.5054	2.7178	8.105	
					III	3.4887	2.7569	8.125	

It only remains to remark that the concentration of hydrogen ions in such solutions as those here concerned is subject to marked alteration on dilution.

<sup>1)</sup> See S. P. L. Sørensen and Margrethe Høyrup l. c. **12**, 169 and **103**, 217.

In N° 1, the undiluted filtrate showed  $p_H = 4.81$ , whereas 10 ccm. of the filtrate diluted to 100 ccm. showed  $p_H = 5.76$ . This agrees very well with the fact that the solutions I, II and III, all of which contained abt. 8 gr. of the precipitate with adherent mother liquor diluted to 100 ccm., showed  $p_H = 5.78, 5.78$  and  $5.77$  respectively.

Similarly, in N° 2, the undiluted filtrate showed  $p_H = 5.48$ , whereas the filtrate diluted to ten times its volume showed  $p_H = 6.19$ . Solutions I, II and III here showed  $p_H = 6.31, 6.27$  and  $6.27$  respectively.

### c. Crystallisation of egg-albumin phosphate at different concentration of hydrogen ions.

The experiment was designed to comprise the preparation of 9 mixtures, each containing the same weight of a given egg-albumin phosphate solution, the same weight of a saturated solution of primary ammonium phosphate and of "secondary", and the same weight (water plus phosphoric acid), but the proportion between water and phosphoric acid and thus the concentration of hydrogen ions, varies in the different experiments. There should thus be in all experiments the same weight of egg-albumin and the same weight of ammonia, while the sum of the weights of water and phosphoric acid also should be the same in all experiments.

If the egg-albumin content in the filtrates after crystallisation were then calculated as aqueous albumin phosphate (by multiplication of the quantity of protein-nitrogen by the factor 1) the filtrates should then — supposing the arrangement of the experiments to be properly adhered to and the value taken for 1 to be correct — show the same ammonia content per 100 gr. (water plus phosphoric acid). The result of the experiments, set forth in Table 2, shows that this also proved to be the case, and the experiments are so far satisfactory.

On the other hand, it was found difficult to get the crystallisation going with the salt-concentrations chosen, and in order to obtain fairly abundant crystallisation it was necessary in all the experimental mixtures to add somewhat more of the "secondary" than originally intended, so that the entire series was shifted over to the alkaline side in such a degree that the con-



Table 2.

Crystallisation of egg-albumin phosphate at different concentrations of hydrogen ions (constant ammonia content but varying proportions between water and phosphoric acid).

Exp. No	100 gr. filtrate contained		Per each 100 gr. water + phosphoric acid (r = 8.12) were found		Concentration of hydrogen ions in the filtrate
	Ammonia N in gr. (a <sub>p</sub> )	Protein N in gr. (P <sub>F</sub> )	Egg albumin phosphate gr.	Ammonia (NH <sub>3</sub> ) gr.	
1	3.9179	0.2515	2.191	5.111	5.22
2	3.9058	0.2055	2.584	5.113	5.25
3	3.8924	0.3222	2.823	5.107	5.28
4	3.8839	0.3652	3.212	5.114	5.31
5	3.8684	0.4202	3.713	5.117	5.34
6	3.8408	0.4941	4.393	5.113	5.38
7	3.8155	0.5600	5.007	5.107	5.41
8	3.7485	0.7581	6.895	5.104	5.48
9	3.7308	0.8230	7.527	5.108	5.53

centration of hydrogen ions in the most acid of the experimental mixtures was less than the optimal concentration of hydrogen ions for crystallisation under the given conditions. It should here be borne in mind, however, that when the ammonia content is kept constant, the variation in concentration of hydrogen ions is accompanied — or rather produced — by a variation in the content of phosphoric acid, and the great difference in the filtrates' content of egg-albumin phosphate is thus doubtless not solely due to difference in the proportion between content of water and phosphoric acid. This proportion is not exactly known for the various mixtures, but the phosphoric acid content was highest in Exp. N<sup>o</sup> 1. In this mixtures, we had something over 20 gr. phosphoric acid per 100 gr. (water plus phosphoric acid) and the phosphoric acid content diminished gradually down through the series, so that in Exp. N<sup>o</sup> 9, the mixture had abt. 1.5 gr. phosphoric acid per 100 gr (water plus phosphoric acid) less than in Exp. 1.

The mixtures were left to crystallise for 3 weeks at ordinary temperature, with a good daily stirring, and afterwards filtered with the usual precautions.

It will be seen from the table, in the first place, that the solubility of the egg-albumin phosphate with a constant concentration of ammonia rises very markedly with decreasing concentration of phosphoric acid — and thus of hydrogen ions. And secondly, the constant or perhaps slightly decreasing value for ammonia content set forth in the last vertical column but one of the table, shows that the value assumed for  $r$  (8.12) is hardly too high.

*April 1923.*

COMPTES-RENDUS

DES TRAVAUX

DU

LABORATOIRE CARLSBERG

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15<sup>ME</sup> VOLUME.

No. 3



COPENHAGUE

EN COMMISSION CHEZ H. HAGERUP

IMPRIMERIE DE THIELE

1923

*Prix: 90 Øre*



# SUR LE DOSAGE DE MÉLANGES DE SACCHAROSE ET DE SUCRE INTERVERTI OU DE LACTOSE

PAR

HANS JESSEN-HANSEN.

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La Délégation Inter-scandinave pour les Produits alimentaires, qui a pour mission, entre autres, d'établir des méthodes d'analyse uniformes pour l'examen des comestibles dans les pays scandinaves, s'est adressée, il y a un peu plus d'un an, au Laboratoire Carlsberg, au sujet de certaines méthodes accompagnées de tables d'analyses, que celui-ci avait publiées en son temps et qui avaient pour objet l'estimation des sucres réducteurs; la Délégation demandait à notre institut de donner à ces méthodes une extension permettant d'en faire application à l'estimation de la composition des mélanges de sucres faisant partie des jus de fruits, des confitures et du lait condensé et qui, par conséquent, font souvent l'objet d'une analyse dans la pratique.

Notre laboratoire a cru devoir satisfaire à cette demande, et c'est l'auteur de la présente notice qui, ayant coopéré à la construction des tables plus anciennes mentionnées ci-dessus, fut chargé d'en élaborer de nouvelles. A vrai dire, si l'on eût pu prévoir les difficultés de toute sorte qui ont surgi au cours de l'exécution de ce travail, il est peut-être douteux qu'on se fût décidé à l'entreprendre.

En effet, dans quelques essais préliminaires faits suivant les préceptes de Kjeldahl<sup>1)</sup>, il s'est trouvé impossible d'obtenir des résultats s'accordant avec les tables établies par ce savant: on trouva dans tous les cas environ  $1/2$  pour cent de cuivre en moins, soit que l'essai considéré portât sur le glucose ou sur le sucre interverti tiré de sucre de canne, quoique des estimations préa-

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<sup>1)</sup> Comptes rendus du Laboratoire de Carlsberg IV (1895).

lables de leur pouvoir rotatoire vis-à-vis de la lumière polarisée eussent montré que les deux sucres devaient être considérés comme parfaitement purs. Par contre, des essais semblables effectués sur du sucre interverti, suivant le procédé de l'auteur de cette notice<sup>1)</sup>, donnèrent des résultats concordants avec la table. Tous ces essais avaient été réalisés à l'aide de réactifs fraîchement préparés. Par hasard, je retrouvai intact dans le laboratoire un faible échantillon restant de la solution de sulfate de cuivre utilisée, il y avait vingt-cinq ans environ, lors de l'élaboration des tables précitées, et quelques essais faits avec cette solution fournirent des résultats qui étaient en parfait accord avec les tables, — c'est-à-dire que les écarts qu'ils montraient n'étaient pas plus grands que ceux des essais sur lesquels les tables étaient basées.

On était ainsi amené à penser que l'erreur était attribuable à la solution cuivrique. Nous avons alors essayé six préparations différentes; le résultat fut toujours le même, c'est-à-dire qu'en procédant suivant l'indication de Kjeldahl prescrivant «50 cc de liqueur de Fehling», on a un peu de cuivre en moins, soit qu'on emploie du glucose ou du sucre interverti, alors qu'au contraire, en suivant le procédé de Jessen-Hansen on a trouvé maintenant à peu près autant de cuivre en plus. Au contraire, il n'y avait point de différence essentielle entre les différentes solutions de cuivre: le résultat était sensiblement le même soit qu'on employât une solution cuivrique préparée au moyen de sulfate cuivrique de Kahlbaum recristallisé plusieurs fois, soit qu'on eût recours à une eau-mère provenant de cette préparation, soit enfin qu'on mit en œuvre un sel dont la solution laissât déposer, par le repos, des quantités appréciables de fer. Quant au sel de Kahlbaum *pro analysi*, il s'est trouvé inutilisable tel quel, parce qu'une dissolution de cette préparation, laissée en repos, fit tomber un dépôt blanchâtre, probablement de quelque sel basique.

Ainsi donc, les sucres employés étaient bien purs, et je me conformais strictement aux préceptes suivis antérieurement; mais néanmoins, j'arrivais à des résultats divergeant de ceux obtenus alors et qui étaient sans nul doute tout aussi corrects que ceux trouvés maintenant. Nous nous sommes alors demandé s'il ne vaudrait pas mieux prendre une voie toute nouvelle. Il y en a

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<sup>1)</sup> Comptes rendus du Laboratoire de Carlsberg IV, 340 (1899).

en effet. Bertrand, par exemple<sup>1)</sup>, fait dissoudre l'oxyde cuivrique déposé dans le sulfate ferrique, qui subit alors une réduction partielle; la partie qui a subi la réduction est titrée au moyen de permanganate de potassium. Maquenne, au contraire<sup>2)</sup>, préfère soumettre directement à la titration dans la liqueur de Fehling le cuivre ayant échappé à la réduction; pour cela, il opère par iodométrie, sans filtration. Il est évident, cependant, que la réaction par laquelle se fait en réalité le dosage du sucre, c'est la réduction du cuivre dans la liqueur alcaline, alors qu'au contraire la manière dont on fait ensuite le dosage de l'oxyde cuivrique déposé est en elle-même tout à fait indifférente, n'étant plutôt qu'une question de commodité. En cas que la réaction du sucre vis-à-vis de la liqueur cuivrique soit influencée par des facteurs inconnus, il va de soi que ces influences ne peuvent être éliminées par aucun traitement subséquent de l'oxyde cuivrique.

Il paraît donc peu probable qu'on puisse obtenir de meilleurs résultats par aucune de ces deux voies: En ce qui concerne celle indiquée par Bertrand, il semble évident que, vu la nécessité de séparer par filtration l'oxyde cuivrique, l'opérateur pourra le peser ou le titrer à son gré<sup>3)</sup>; dans l'un et l'autre cas, on peut naturellement se servir des mêmes tables. Quant à la manière d'agir préconisée par Maquenne, elle ne conduira pas non plus au but parce que, de son aveu même, elle n'offre un avantage sur celle de Bertrand que lorsqu'il s'agit de minimes doses de cuivre, par exemple dans le cas de la détermination d'une trace de sucre interverti dans les sucres de canne du commerce.

Comment donc faire? — Il y a ici lieu de faire remarquer que d'autres chimistes se sont heurtés à des difficultés semblables. C'est ainsi que Maquenne dit textuellement<sup>4)</sup>:

«Les tables doivent être naturellement construites pour chaque cas et par l'opérateur même, qui doit s'en servir; nous estimons, en effet, que de pareils recueils de chiffres, fondés sur des bases

1) Bulletin de la Société chimique [3]. 35, 1285 (1906).

2) La dernière fois, *ibid.* (4) 31, p. 799 (1922).

3) Il peut arriver, dans l'analyse de certains produits naturels, que l'oxyde cuivrique se dépose sous forme de grains tellement fins que la filtration à travers les tubes d'asbeste ordinaires et, par suite, la pesée etc. deviennent très pénibles; en ce cas, il peut y avoir avantage à suivre le procédé de Bertrand, qui permet d'utiliser des filtres présentant une surface filtrante beaucoup plus grande.

4) Comptes rendus 162, p. 212.

essentiellement empiriques et dont quelques-unes sont de nature toute personnelle, n'ont de réelle valeur que pour celui-là même qui les a établis ou calculés. C'est pourquoi nous nous dispenserons de publier les nôtres. — — — — Le degré d'exactitude auquel on peut ainsi prétendre en travail courant ne paraît guère pouvoir dépasser  $\frac{1}{50}$  du poids des réducteurs cherchés.»

D'après les considérations précédentes, il semblerait qu'il y aurait lieu d'approuver les remarques de Maquenne que nous venons de citer, et de laisser à chaque expérimentateur le soin d'imaginer son mode opératoire et de construire ses tables. Cependant, il serait sans doute impraticable à chaque analyste qui en aurait besoin d'élaborer, dans chaque cas particulier, de pareilles tables à son propre usage; ou bien elles ne seraient pas construites avec l'exactitude indispensablement nécessaire, de sorte que, loin d'atteindre au degré de précision plus élevé qu'il se serait proposé d'obtenir, il n'aboutirait en définitive qu'à une précision moindre. C'est pourquoi nous nous sommes décidé à construire les tables données à la fin du présent mémoire.

Dans ce qui va suivre, nous allons rendre compte des principes sur lesquels nous nous sommes guidé dans leur élaboration, de même qu'on trouvera expliquée la manière dont il conviendra de les utiliser.

Ainsi que nous l'avons dit, il s'agit d'établir la composition d'un mélange de sucre de canne et de l'un des sucres réducteurs, sucre interverti ou lactose, et cela à l'aide de la quantité de cuivre qui dans des conditions données se dépose dans la liqueur de Fehling. Comme il a été indiqué aussi, ce problème présente une double difficulté: Chauffé en présence d'une solution alcaline de cuivre, le sucre de canne, lui aussi, laisse déposer de l'oxyde cuivrique, en quantité de beaucoup inférieure, il est vrai, à celle déposée par les deux autres sucres (dans des conditions expérimentales convenables, elle peut être réduite pratiquement au minimum), mais qu'on peut difficilement réduire à zéro. Et, d'autre part, la quantité d'oxyde cuivrique que fait déposer un mélange de sucre de canne et d'un sucre réducteur, ou bien de deux sucres réducteurs, n'est point égale à la somme des quantités que donnerait chacun, isolément, des constituants du mélange.



Le seul moyen qui nous reste, sera donc de construire des tables permettant de trouver quelle est, de l'un des deux sucres, la quantité qui correspond à une quantité donnée de cuivre, quand ce sucre se trouve à côté d'une certaine quantité de l'autre. Lorsqu'on procédera alors à l'analyse du mélange, il conviendra, par des essais préliminaires, de s'arranger de manière à évaluer d'abord — de façon approchée — le taux de l'un des deux sucres, pour doser ensuite l'autre, à l'aide d'un essai où l'on opère justement sur la quantité maintenant connue du premier sucre et indiquée par la table; éventuellement, on pourra se servir de cette dernière donnée comme point de départ d'un dosage plus précis du premier sucre.

Vu, du reste, que la réaction que nous utilisons ici, savoir l'oxydation des sucres au moyen d'une solution cuivrique alcaline, loin de marcher d'après des relations stoechiométriques bien définies, se trouve dans une grande mesure sous la dépendance des conditions expérimentales actuelles, c'est-à-dire de la composition et de la concentration du mélange à réaction, du temps de chauffage et de la température, il s'en suit qu'aucune table ne sera utilisable que sous les valeurs bien définies de ces facteurs pour lesquelles elle aura été établie.

Voici les conditions expérimentales dans lesquelles ont été construites les tables qui accompagnent ce mémoire:

Dans une fiole conique de 150 cm<sup>3</sup> de capacité, on mélange, immédiatement avant le commencement de l'expérience, 8<sup>gr</sup>, 65 de sel de Seignette avec 25 cm<sup>3</sup> d'une solution de soude normale à 3.25 et 25 cm<sup>3</sup> d'une solution de sulfate de cuivre contenant 69<sup>gr</sup>.278 de CuSO<sub>4</sub>, 5 *aq*, soit 17<sup>gr</sup>.592 de Cu dans un litre. On y ajoute la solution sucrée, puis l'on complète avec de l'eau jusqu'au volume de 100 cm<sup>3</sup>, indiqué par un trait au côté de la fiole.

La fiole est munie d'un bouchon percé de deux trous, par l'un desquels passe un tube de verre descendant à peu près jusqu'au fond, tandis que l'autre est traversé par un tube pareil qui s'ouvre un peu au-dessous du bouchon. On fait passer dans la fiole un courant d'hydrogène jusqu'à ce que l'air atmosphérique en soit totalement expulsé, ce qui prend trois minutes environ. Ensuite, on met la fiole dans un bain-marie bouillant, de façon telle que les surfaces des deux liquides soient sensiblement au même niveau. On laisse la fiole dans l'eau pendant 5 minutes

bien exactement, en contrôlant le temps à l'aide d'une montre à secondes. Le bain-marie doit être assez grand pour que l'eau ne cesse pas de bouillir lorsqu'on y met la fiole.

Les 5 minutes écoulées, on retire la fiole du bain-marie, pour en filtrer aussitôt le contenu, encore chaud, à la trompe, à travers un tube d'asbeste Allihn. On soumet l'oxyde cuivreux précipité et la fiole à un lavage soigneux à l'eau bouillie, encore chaude, et à l'alcool, en ayant soin de retirer de la fiole la totalité de l'oxyde cuivreux; en effet, on éprouve parfois quelque difficulté à en détacher la dernière trace. Finalement, le contenu du tube est lavé à l'esprit-de-vin, puis avec un peu d'éther, qu'il faudra enlever soigneusement en suçant, après quoi le tube, sans être séché davantage, est placé dans un bain d'air en tôle, chauffé au moyen d'un bec de Bunsen; en même temps, on fait passer à travers le tube un courant d'hydrogène sec. (Si l'hydrogène renferme une forte quantité d'hydrogène arsenié, il est bon de le purifier en le conduisant à travers une ou deux bouteilles de lavage contenant une dissolution de nitrate d'argent dans l'acide nitrique<sup>1)</sup>; un peu d'arsène ne gêne pas.) Une fois l'oxyde cuivreux réduit en cuivre — ce qu'on peut reconnaître aisément à la couleur —, on fera refroidir le tube dans le courant d'hydrogène et, après au moins dix minutes de séjour à la balance ou sous l'exsiccateur, on le pèse.

Les tubes dont on se sert doivent être nettoyés au moyen d'acide nitrique, puis lavés et séchés dans un courant d'hydrogène, comme on le fait pour une analyse. Ils ne doivent perdre, à chaque utilisation, que 0<sup>m</sup><sup>sr</sup>,8, en moyenne. — Ils se préparent facilement de la façon suivante: Au fond d'un tube Allihn de bon verre, on met d'abord un peu de coton de verre, puis une couche, épaisse d'un centimètre environ, d'asbeste inflexible tel que celui utilisé pour les creusets de Gooch et auquel, en le délayant dans l'eau, on aura enlevé à la fois les parties les plus déliées et les plus grossières.

Si nous avons choisi une durée de chauffage de 5 minutes seulement, au lieu des 20 minutes prescrites par Kjeldahl<sup>2)</sup>, c'est parce qu'ainsi on réduit au minimum la réduction du saccharose, tout en permettant aux sucres réducteurs proprement dits d'exercer de beaucoup la plus grande partie de l'effet qu'ils sont capables

<sup>1)</sup> Gmelin-Kraut: Handbuch der anorg. Chemie, 7 Aufl. III, 2, p. 437.

<sup>2)</sup> A l'endroit cité.

de produire, en sorte que cette diminution de chauffe ne nuit en rien à la précision nécessaire, pourvu qu'on observe le temps le plus exactement possible. — Quant au chauffage au bain-marie, que j'ai cru devoir préférer au procédé adopté par la plupart des expérimentateurs — entre aux, Bertrand et Maquenne —, et qui consiste à faire bouillir sur une toile métallique à l'aide d'une lampe, je suis d'avis que ce procédé-la se prête mieux à être reproduit que ne le fait l'ébullition à feu nu.

En opérant de la manière que nous venons d'exposer, nous avons effectué les cinq séries d'expériences que nous allons relater.

1°. Sucre interverti pur. Ayant pesé 0<sup>gr</sup>,950 de saccharose, je l'ai fait passer, à l'aide d'environ 70 cm<sup>3</sup> d'eau, dans une fiole pesée de la capacité de 100 cm<sup>3</sup>. Après y avoir ajouté 10 cm<sup>3</sup> d'acide chlorhydrique norm. au 1/5, on a placé la fiole dans un bain-marie bouillant de manière à ce que le niveau du mélange fût sensiblement le même que celui de l'eau environnante. Après trente minutes de séjour dans l'eau bouillante, la fiole fut retirée, refroidie sous le robinet à eau, additionnée de 10 cm<sup>3</sup> de soude norm. au 1/5, remplie d'eau jusqu'au trait, et enfin pesée (*Interversion d'après Nicol*). On fit le dosage de la quantité de cuivre réduit, dans les conditions expérimentales indiquées plus haut, par des quantités pesées de cette solution à 1 pour cent de sucre interverti. En partant des résultats numériques ainsi obtenus, on a calculé, au moyen de la méthode des moindres carrés, les coefficients,  $\alpha$ ,  $b$  et  $c$ , d'une équation de cette forme:

$$\text{Cu} = \alpha + bI \div cI^2,$$

équation qui conséquemment exprime la dépendance réciproque existant, dans les conditions de l'expérience, entre le sucre interverti et le cuivre.

Après substitution des valeurs trouvées, on a:

$$\text{Cu} = 0.20 + 1.97122 I \div 0.000735 I^2.$$

Dans le Tableau 1 ci-contre, on trouvera inscrites tant les valeurs trouvées d'après lesquelles l'équation a été calculée, que les quantités de Cu qui, selon cette équation, correspondraient au sucre pesé d'avance.

Tableau 1.

Sucre interverti pur.

$$\text{Cu} = 0.20 + 1.97122 \text{ I} \div 0.000735 \text{ I}^2.$$

Sucre	Cu		T.—C.
	Trouvé	Calculé	
0	0.1	0.20	— 0.1 )
0	0.2	0.20	— 0.0 )
9.92	19.9	19.68	+ 0.22
20.12	40.5	39.56	+ 0.94
20.04	39.4	39.40	± 0.00
49.87	96.2	96.67	÷ 0.47
50.08	95.6	97.08	÷ 1.48
73.72	140.6	141.53	÷ 0.93
99.39	189.5	188.86	+ 0.64
99.47	190.1	189.01	+ 1.09
99.44	189.2	188.95	+ 0.25
99.58	189.0	189.20	÷ 0.20
150.12	279.9	279.56	+ 0.34
198.39	363.2	362.34	+ 0.86
198.80	361.8	363.03	÷ 1.23
199.35	363.5	363.93	÷ 0.43
217.39	394.4	393.99	+ 0.41
222.26	401.9	402.01	÷ 0.11

Les deux premiers essais (essais témoins) ont été laissés de côté dans le calcul de l'équation.

2° Sucre interverti mélangé de saccharose. On a préparé, à la manière décrite plus haut, une solution à 1 pour cent de sucre interverti et, en même temps, une solution de saccharose, également à 1 p. c. Ensuite, à l'aide de deux burettes, on a mesuré, pour chaque essai, de telles quantités de ces deux solutions, que leur somme se montât toujours à 24 cm<sup>3</sup>; en même temps, la solution de sucre interverti fut pesée et la quantité de cuivre réduit par ce mélange fut dosée, après quoi on soumit les résultats obtenus au même calcul que ci-dessus.

On a :

$$\text{Cu} = 1.94 + 1.97755 \text{ I} \div 0.000794 \text{ I}^2.$$

Voir d'ailleurs le Tableau 2 ci-contre.

Tableau 2.

Saccharose + sucre interverti = 0<sup>gr.</sup>240.

$$\text{Cu} = 1.94 + 1.97755 \text{ I} \div 0.000794 \text{ I}^2.$$

Sucre interverti	Cu		T.—C.
	Trouvé	Calculé	
0	1.8	1.94	÷ 0.14
0	1.7	1.94	÷ 0.24
( 10.08	21.9	21.79	+ 0.11
20.07	42.3	41.31	+ 0.99
40.20	80.6	80.14	+ 0.46
59.35	115.3	116.51	÷ 1.21
79.34	153.7	153.84	÷ 0.14
( 80.39	153.1	155.79	÷ 2.69
( 99.33	187.2	190.53	÷ 3.33
99.42	190.4	190.68	÷ 0.28
119.31	226.8	226.57	+ 0.23
139.59	262.5	262.51	÷ 0.01
( 139.59	262.0	262.51	÷ 0.51
160.43	298.1	298.75	÷ 0.65
181.00	334.1	333.85	+ 0.25
198.70	364.2	363.51	+ 0.69
218.82	397.5	396.63	+ 0.87
239.17	428.7	429.47	÷ 0.77
238.61	428.0	428.57	÷ 0.57

Les quatre essais mis entre parenthèses n'ont pas été utilisés dans le calcul de l'équation.

3° Lactose pur. Bien que le mode de dosage du lactose pur ait déjà été indiqué par Kjeldahl<sup>1)</sup>, j'ai cru qu'il y aurait intérêt à pouvoir doser ce sucre suivant la même technique que celle dont il convient de se servir lorsqu'il fait partie d'un mélange; c'est pourquoi nous avons construit le Tableau 3 sur le même principe que les deux précédents.

4° Lactose mélange de saccharose. On a préparé une solution à 1 p. c. de lactose et une solution à 2 p. c. de saccharose, puis on a déterminé la quantité de cuivre qui serait réduite par  $10 \text{ cm}^3 = 200 \text{ mgr.}$  de saccharose et des quantités variables,

<sup>1)</sup> A l'endroit cité.

Tableau 3.

Lactose pur.

$$\text{Cu} = 0.58 + 1.25431 \text{ M} \div 0.000008784 \text{ M}^2.$$

Lactose pur	Cu		T — C.
	Trouvé	Calculé	
0	0.1	0.58	$\div 0.48$
0	0.2	0.58	$\div 0.38$
15.34	19.5	19.82	$\div 0.32$
19.77	25.2	25.38	$\div 0.18$
22.59	27.9	28.91	$\div 1.01$
48.80	61.0	61.77	$\div 0.77$
52.66	67.4	66.61	$+ 0.79$
101.14	127.7	127.35	$+ 0.35$
147.71	186.5	185.66	$+ 0.84$
149.71	188.3	188.17	$+ 0.13$
202.38	253.0	254.07	$\div 1.07$
204.65	256.9	256.91	$\div 0.01$
249.12	310.4	312.51	$\div 2.11$
296.40	373.2	371.59	$+ 1.61$
298.36	372.9	374.04	$\div 1.14$
325.91	410.6	408.44	$+ 2.16$
327.99	411.7	411.04	$+ 0.66$
(328.29)	413.8	411.41	$+ 2.39)$
331.38	417.4	415.27	$+ 2.13$
(346.07)	436.5	433.61	$+ 2.89)$
348.59	434.9	436.76	$\div 1.86$
349.86	436.5	438.34	$\div 1.84$

Les deux essais mis entre parenthèses n'ont pas été utilisés dans le calcul de l'équation.

pesées, de la solution de lactose. Les résultats ainsi obtenus servirent à calculer, de la manière indiquée plus haut, l'équation ci-dessous, ainsi que le Tableau 4 qui s'y rattache.

5° Lactose mélangé de sucre interverti. On a préparé une solution de lactose à  $\frac{1}{2}$  p. c. et une solution de sucre interverti à 1 p. c. On a déterminé la quantité de cuivre réduite par 10 cm<sup>3</sup> de la solution de lactose = 50 mgr. de lactose + des quantités variables, pesées, de la solution de sucre interverti.

Tableau 4.

0.200 gr. Saccharose + Lactose.

$$Cu = 1.94 + 1.25970 M \div 0.00000025 M^2.$$

Lactose	Cu		T.—C.
	Trouvé	Calculé	
0	1.2	1.94	$\div 0.74$
20.26	27.6	27.46	$+ 0.14$
21.04	27.3	28.43	$\div 1.13$
51.61	68.2	66.95	$+ 1.25$
97.35	125.2	124.56	$+ 0.64$
100.99	129.5	129.14	$+ 0.36$
148.87	192.2	189.44	$+ 2.76$
200.70	254.6	254.73	$\div 0.13$
204.86	258.7	259.97	$\div 1.27$
249.64	316.6	316.37	$+ 0.33$
252.06	316.8	319.42	$\div 2.62$
293.83	371.9	372.01	$\div 0.11$
300.87	380.8	380.90	$\div 0.11$
324.05	411.0	410.09	$+ 0.91$
328.54	416.6	415.75	$+ 0.85$

Les résultats servirent à calculer l'équation ci-dessous et le Tableau 5 qui s'y rattache.

Les Tables I à V annexées à ce mémoire contiennent les solutions des cinq équations ci-dessus pour toutes les valeurs de cuivre, exprimées en milligrammes entiers, qui peuvent se présenter dans les conditions de ces expériences.

Le sucre de canne employé dans nos recherches avait été recristallisé à plusieurs reprises dans de l'alcool à 85 p. c. Le pouvoir rotatoire qu'il accusait vis-à-vis de la lumière polarisée, était représenté pour la lumière de sodium par  $[\alpha]_D = 66^\circ,43 - 66^\circ,48$ , et pour la lumière jaune de mercure,  $\lambda = 579$ , par  $[\alpha]_{Hg} = 69^\circ,204 - 69^\circ,205$ . Les observations étaient faites dans des tubes de 400 mm à  $20^\circ$  et avec une concentration de 20 % env. — Landolt<sup>1)</sup>

<sup>1)</sup> Das optische Drehungsvermögen, 2 Aufl. 530 (1898).

Tableau 5.

50 mgr. Lactose + Sucre interverti.

$$\text{Cu} = 64.91 + 1.807912 \text{ I} \div 0.00050226 \text{ I}^2.$$

Sucre interverti	Cu		T.-C.
	Trouvé	Calculé	
0	63.5	64.91	$\div 1.41$
0	64.0	64.91	$\div 0.91$
19.36	100.0	101.46	$\div 1.46$
21.22	106.0	104.95	$+ 1.05$
40.61	142.4	141.15	$+ 1.25$
42.84	146.0	145.30	$+ 0.70$
59.97	177.1	176.92	$+ 0.18$
62.61	182.0	181.77	$+ 0.23$
79.91	214.5	213.56	$+ 0.94$
82.66	217.9	218.36	$\div 0.46$
98.75	247.8	247.43	$+ 0.37$
102.62	254.8	254.38	$+ 0.42$
118.14	283.3	282.12	$+ 1.18$
121.41	288.0	287.94	$+ 0.06$
137.42	316.5	316.24	$+ 0.26$
138.52	317.4	318.17	$\div 0.77$
156.97	349.9	350.45	$\div 0.55$
157.95	351.4	352.16	$\div 0.76$
177.57	386.1	386.08	$+ 0.02$
176.16	384.4	383.66	$+ 0.74$
197.35	419.3	419.90	$\div 0.60$
197.75	419.1	420.58	$\div 1.48$

$[\alpha]_D = 66^\circ,48$ . A partir des déterminations de Pellat<sup>1)</sup>, on fait l'interpolation de  $[\alpha]_{H_2} = 69^\circ,026$ , et de celles de Seyffahrt<sup>2)</sup>  $69^\circ,312$ .

Le lactose était un produit technique de parfaite pureté, qu'on a recristallisé plusieurs fois dans l'eau de façon telle que les cristaux ne se déposent qu'à la température ordinaire du laboratoire. Ils étaient séchés à l'air, puis sous l'exsiccateur sur

<sup>1)</sup> Zeitschrift d. Ver. d. Zucker-Industrie **51**, 831 (1901).

<sup>2)</sup> Wiedemanns Annalen **41**, 128 (1890).



l'acide sulfurique jusqu'à poids constant. Dans des tubes de 400 mm à 20° et à une concentration de 10 p. c. env., on trouva :

$$[\alpha]_D = 52^{\circ},44-52^{\circ},52 \text{ et } [\alpha]_{Hg} = 54^{\circ},81-54^{\circ},94.$$

Schmöger<sup>1)</sup>  $[\alpha]_D = 52^{\circ},53$ . Grossmann et Bloch  $[\alpha]_D = 52^{\circ},42-52^{\circ},50$ . En partant des déterminations de Grossmann et de Bloch<sup>2)</sup>, on a interpolé  $[\alpha]_{Hg} = 54^{\circ},27$ . Attendu que cette dernière valeur a été interpolée à partir de déterminations faites dans des solutions assez faibles ( $C = 2$ ), on peut penser que la valeur ici trouvée est meilleure, et que le saccharose aussi bien que le lactose ont été à l'état de pureté.

Si l'on compare, d'un côté, les Tables I et III, relatives aux sucre interverti et lactose purs, et, de l'autre, les Tables II et IV, concernant des mélanges de ces sucres avec du saccharose, il pourra sembler à première vue que la divergence soit tellement insignifiante qu'il serait justifié de ne point tenir compte de l'effet produit par le saccharose, autrement dit, qu'on puisse se contenter des Tables I et III. Je crois cependant, qu'en réalité il n'en est pas ainsi. D'abord, pour cette raison d'ordre purement théorique qu'il ne faut jamais, pour peu qu'il y ait moyen de l'éviter, employer une méthode entachée d'un défaut systématique, si léger qu'il soit; et puis, il faut bien remarquer que, dans les cas où le saccharose est présent en quantité relativement importante, l'erreur que l'on commettrait en négligeant d'en tenir compte dans les calculs ne serait pas tout à fait sans conséquence; d'ailleurs, une fois les tables établies, il n'est guère plus difficile de les utiliser toutes les deux que de n'en employer qu'une.

Pour ce qui regarde le degré de précision qu'on pourra atteindre en utilisant ces tables, il est à remarquer que les écarts constatés entre les expériences sur lesquelles reposent les tables et, de l'autre côté, les résultats calculés à l'aide des équations, n'atteignent pas, en moyenne,  $1/2$  p. c. et ne dépassent 1 p. c. que dans des cas exceptionnels. En tenant compte de ce fait et de ce que nous avons dit au début au sujet du degré de concordance réalisable avec les déterminations d'autrefois, on conviendra, je

<sup>1)</sup> Berichte der d. ch. Ges. **13**, p. 1922 (1888).

<sup>2)</sup> Zeitschrift d. Ver. d. Zucker-Industrie, **62**, 62 (1912).

pense, qu'on ne saurait guère être sûr d'obtenir un degré d'exactitude supérieur à celui indiqué par Maquenne (2 p. c.), bien que dans beaucoup de cas, peut-être même le plus souvent, les résultats puissent se rapprocher davantage de la vérité, si l'on opère avec tous les soins requis. Pourtant, quant à garantir, comme le font H. R. Riiber et C. N. Riiber<sup>1</sup>), une précision de 0.5 p. c. de la quantité de sucre trouvée, j'estime qu'il faut en dissuader les expérimentateurs; car »à l'impossible nul n'est tenu«.

### Mode de se servir des Tables.

Quand la substance à examiner est constituée par un mélange de sucre interverti et de sucre de canne, on en préparera une solution à 2 p. c. environ, suivant les données actuelles, ou bien d'après un essai préalable.

On prendra 50 cm<sup>3</sup> de cette solution pour en effectuer l'inversion suivant Nicol, c'est-à-dire qu'on y ajoute 20 cm<sup>3</sup> de HCl norm. au  $\frac{1}{10}$ , ou bien une dose y correspondante d'acide plus fort et d'eau. On chauffe au bain-marie bouillant pendant une demi-heure, en y plongeant la fiole renfermant le mélange, de façon à ce que le niveau de ce dernier soit le même que celui du bain d'eau. La demi-heure écoulée, on refroidit la solution sous le robinet à eau jusqu'à la température du laboratoire, pour ajouter alors une quantité de soude équivalente à celle de l'acide employé. Après avoir complété la solution à 100 cm<sup>3</sup>, on fera le dosage du sucre interverti =  $a$  à l'aide de la Table I, et on aura la somme du sucre interverti primitif + celui susceptible d'être formé par le sucre de canne présent.

Un nouvel essai fait sur la solution primitive en quantité telle que, selon l'estimation effectuée, elle corresponde à 240 mgr. de sucre interverti, donnera alors, à l'aide de la Table II, le taux du sucre interverti primitif  $b$ , d'où l'on aura pour celui du sucre de canne:  $(a \div b) 0.95$ .

Quand le mélange qu'il s'agit d'analyser est formé de lactose et de sucre de canne, il faut constater de façon approchée, en consultant la Table IV, la teneur en lactose. Ensuite, on prépare une solution de lactose à 1 p. c. env., et de celle-ci on fait intervertir 50 cm<sup>3</sup> d'après Nicol, comme décrit ci-dessus;

<sup>1</sup> Fresenius: Zeitschrift 40. p. 98 1901.

puis on neutralise et complète à  $100 \text{ cm}^3$ , pour déterminer alors la quantité de cuivre qui sera réduite par  $10 \text{ cm}^3$  de cette solution, dont la teneur en lactose est d'une cinquantaine de mgr. La Table V permettra ensuite de trouver une valeur approximative du sucre interverti et, conséquemment, du saccharose contenus dans la solution primitive.

Afin d'obtenir les valeurs exactes, on prend alors, de la solution primitive, une portion contenant, d'après la seconde détermination, précisément 200 mgr. de saccharose, et l'on y détermine, à l'aide de la Table IV, le taux exact du lactose. Et une portion de solution intervertie contenant selon ce dosage-ci 50 mg. de lactose donnera alors, par l'emploi de la Table V, la vraie teneur en saccharose interverti.

### Exemples.

#### Saccharose et Sucre interverti.

$1^{\text{er}}$ ,900 de saccharose ont été intervertis suivant le précepte. La solution fut additionnée encore de  $2^{\text{es}}$ ,000 de saccharose, puis complétée à  $100 \text{ cm}^3 = 101^{\text{es}}$ ,2793, qui contenaient ainsi  $2^{\text{es}}$ ,000 de sucre interverti et une égale quantité de saccharose.

$50 \text{ cm}^3 = 50^{\text{es}}$ ,412 de cette solution furent intervertis suivant le précepte, puis complétés à  $100 \text{ cm}^3 = 101^{\text{es}}$ ,1400.

$1^{\circ}$   $8^{\text{es}}$ ,727 de la solution intervertie fournirent  $323^{\text{mgr}}$ ,2 de Cu, quantité qui selon la Table I correspond à  $175^{\text{mgr}}$ ,3 de sucre interverti. En conséquence, on prend  $6 \text{ cm}^3$  de la solution primitive =  $6^{\text{es}}$ ,186, qui donnent  $230^{\text{mgr}}$ ,2 de Cu, correspondant d'après la Table II à  $121^{\text{mgr}}$ ,3 de sucre interverti.

Ces résultats donnent pour la solution primitive:

$1^{\text{es}}$ ,9831 de sucre inverti avec une erreur se chiffrant par  $\div 0.84 \text{ p. c.}$   
 $1^{\text{es}}$ ,9937 de saccharose — — —  $\div 0.32$  —

$2^{\circ}$   $9^{\text{es}}$ ,9025 de la solution intervertie fournirent  $364^{\text{mgr}}$ ,0 de Cu, correspondant d'après la Table I à  $199^{\text{mgr}}$ ,4 de sucre interverti; et  $6 \text{ cm}^3 = 6^{\text{es}}$ ,1475 de solution primitive donnèrent  $229^{\text{mgr}}$ ,1 de Cu, correspondant d'après la Table II à  $120^{\text{mgr}}$ ,8 de sucre interverti, ce qui dans la solution primitive donne:

$1^{\text{es}}$ ,990 sucre interverti, erreur  $\div 0.50 \text{ p. c.}$   
 $1^{\text{es}}$ ,9895 — saccharose, —  $\div 0.55$  —

## Saccharose + Lactose.

1° 2<sup>gr</sup>,000 de lactose + 2<sup>gr</sup>,000 de saccharose furent dissous, le volume de la dissolution étant de 100 cm<sup>3</sup> = 101<sup>gr</sup>,8462.

Trouvé:

1<sup>gr</sup>,977 de lactose

2<sup>gr</sup>,006 - saccharose,

car 15 cm<sup>3</sup> = 15<sup>gr</sup>,1635 de cette solution fournirent 377<sup>mgr</sup>,3 de Cu, ce qui selon la Table IV correspond à 298<sup>mgr</sup>,0 de lactose.

50 cm<sup>3</sup> = 50<sup>gr</sup>,050 furent intervertis d'après le précepte, puis complétés à 100 cm<sup>3</sup> = 100<sup>gr</sup>,7115, dont 5 cm<sup>3</sup> = 5<sup>gr</sup>,1135, qui selon le premier dosage contiennent 49<sup>mgr</sup>,94 de lactose, fournirent 162<sup>mgr</sup>,7 de Cu, ce qui d'après la Table V correspond à 52<sup>mgr</sup>,3 de sucre inverti. D'après cela, 10 cm<sup>3</sup> de la solution primitive contiennent 200<sup>mgr</sup>,0 de saccharose, et 10 cm<sup>3</sup> = 10<sup>gr</sup>,0715 ont donné 248<sup>mgr</sup>,2 de Cu, ce qui d'après la Table IV correspond à 195<sup>mgr</sup>,5 de lactose ou, en tout, 1<sup>gr</sup>,977 de lactose. donc une erreur de 1,15 p. c. en moins, par rapport à la quantité pesée. En tenant compte de ce fait en calculant le saccharose, on constate qu'au moment du dosage de celui le taux du lactose ne s'est élevé qu'à 49<sup>mgr</sup>,32 au lieu des 50<sup>mgr</sup>,0 prévus par la théorie. En conséquence, vu que 1,25 de sucre interverti donne à peu de chose près le même taux de cuivre que celui fourni par 2 de lactose, il sera nécessaire d'appliquer au dosage du sucre interverti une correction de + 0<sup>mgr</sup>,4, de sorte que le résultat sera 52<sup>mgr</sup>,7 au lieu de 52<sup>mgr</sup>,3, ce qui donne une teneur en saccharose de 2<sup>gr</sup>,006, donc 0,3 p. c. de trop.

2° Pesé 1<sup>gr</sup>,500 de lactose + 2<sup>gr</sup>,500 de saccharose.

Trouvé:

1<sup>gr</sup>,487 de lactose, erreur ÷ 1 p. c.

2<sup>gr</sup>,526 - saccharose, — ÷ 1 —

Table I.

Sucre interverti pur.

$$\text{Cu} = 0.2 + 1.97122 \text{ S} - 0.000735 \text{ S}^2.$$

Cu	0	1	2	3	4	5	6	7	8	9
10	5.1	5.4	5.6	5.9	6.1	6.6	7.1	7.6	8.1	8.6
20	10.10	10.6	11.1	11.6	12.1	12.7	13.2	13.7	14.2	14.7
30	15.21	15.7	16.2	16.7	17.3	17.8	18.3	18.8	19.3	19.8
40	20.35	20.9	21.4	21.9	22.4	22.9	23.5	24.0	24.5	25.0
50	25.52	26.0	26.6	27.1	27.6	28.1	28.6	29.1	29.7	30.2
60	30.69	31.2	31.7	32.3	32.8	33.3	33.8	34.3	34.9	35.4
70	35.89	36.4	36.9	37.5	38.0	38.5	39.0	39.5	40.0	40.6
80	41.11	41.6	42.2	42.7	43.2	43.7	44.3	44.8	45.3	45.8
90	46.36	46.9	47.4	47.9	48.5	49.0	49.5	50.0	50.6	51.1
100	51.62	52.1	52.7	53.2	53.7	54.3	54.8	55.3	55.9	56.4
110	56.91	57.4	58.0	58.5	59.0	59.6	60.1	60.6	61.2	61.7
120	62.22	62.8	63.3	63.8	64.4	64.9	65.4	66.0	66.5	67.0
130	67.55	68.1	68.6	69.2	69.7	70.2	70.8	71.3	71.8	72.4
140	72.90	73.4	74.0	74.5	75.1	75.6	76.1	76.7	77.2	77.7
150	78.28	78.8	79.4	79.9	80.4	81.0	81.5	82.1	82.6	83.1
160	83.68	84.2	84.8	85.3	85.8	86.4	86.9	87.5	88.0	88.6
170	89.10	89.6	90.2	90.7	91.3	91.8	92.4	92.9	93.5	94.0
180	94.55	95.1	95.6	96.2	96.7	97.3	97.8	98.4	98.9	99.5
190	100.02	100.6	101.1	101.7	102.2	102.8	103.3	103.9	104.4	105.0
200	105.51	106.1	106.6	107.2	107.7	108.3	108.8	109.4	109.9	110.5
210	111.03	111.6	112.1	112.7	113.2	113.8	114.4	114.9	115.5	116.0
220	116.57	117.1	117.7	118.2	118.8	119.4	119.9	120.5	121.0	121.6
230	122.14	122.7	123.3	123.8	124.4	124.9	125.5	126.1	126.6	127.2
240	127.74	128.3	128.9	129.4	130.0	130.6	131.1	131.7	132.2	132.8
250	133.36	133.9	134.5	135.1	135.6	136.2	136.7	137.3	137.9	138.4
260	139.00	139.6	140.1	140.7	141.3	141.8	142.4	143.0	143.5	144.1
270	144.67	145.2	145.8	146.4	147.0	147.5	148.1	148.7	149.2	149.8
280	150.37	150.9	151.5	152.1	152.7	153.2	153.8	154.4	155.0	155.5
290	156.10	156.7	157.3	157.8	158.4	159.0	159.6	160.1	160.7	161.3
300	161.86	162.4	163.0	163.6	164.2	164.8	165.3	165.9	166.5	167.2
310	167.64	168.2	168.8	169.4	170.0	170.5	171.1	171.7	172.3	172.9
320	173.45	174.0	174.6	175.2	175.8	176.4	177.0	177.5	178.1	178.7
330	179.29	179.9	180.5	181.1	181.6	182.2	182.8	183.4	184.0	184.6
340	185.17	185.8	186.4	186.9	187.5	188.1	188.7	189.3	189.9	190.5
350	191.07	191.7	192.3	192.8	193.4	194.0	194.6	195.2	195.8	196.4
360	197.00	197.6	198.2	198.8	199.4	200.0	200.6	201.2	201.8	202.4
370	202.96	203.6	204.2	204.8	205.4	206.0	206.6	207.2	207.8	208.4
380	208.95	209.6	210.2	210.8	211.4	212.0	212.6	213.2	213.8	214.4
390	214.98	215.6	216.2	216.8	217.4	218.0	218.6	219.2	219.8	220.4
400	221.04	221.6	222.3	222.9	223.5	224.1	224.7	225.3	225.9	226.5
410	227.13	227.7	228.4	229.0	229.6	230.2	230.8	231.4	232.0	232.6
420	233.25	233.9	234.5	235.1	235.7	236.3	236.9	237.6	238.2	238.8
430	239.41	240.0	240.6	241.3	241.9	242.5	243.1	243.7	244.4	245.0

Table II.

$$\text{Saccharose} + \text{Sucre interverti} = 240 \text{ mgr.}$$

$$Cu = 1.94 \pm 1.97755 \text{ S} \div 0.00079444 \text{ S}^2$$

C	0	1	2	3	4	5	6	7	8	9
0			0.4	0.9	1.3	1.8	2.2	2.7	3.2	3.6
10	4.1	4.6	5.1	5.6	6.1	6.6	7.1	7.6	8.2	8.7
20	9.2	9.7	10.2	10.7	11.2	11.7	12.2	12.7	13.3	13.8
30	14.3	14.8	15.3	15.8	16.3	16.8	17.3	17.9	18.4	18.9
40	19.4	19.9	20.4	20.9	21.5	22.0	22.5	23.0	23.5	24.0
50	24.5	25.1	25.6	26.1	26.6	27.1	27.6	28.2	28.7	29.2
60	29.7	30.2	30.8	31.3	31.8	32.3	32.8	33.3	33.9	34.4
70	34.9	35.4	35.9	36.5	37.0	37.5	38.0	38.6	39.1	39.6
80	40.1	40.6	41.2	41.7	42.2	42.7	43.3	43.8	44.3	44.8
90	45.4	45.9	46.4	46.9	47.5	48.0	48.5	49.0	49.6	50.1
100	50.6	51.1	51.7	52.2	52.7	53.3	53.8	54.3	54.8	55.4
110	55.9	56.4	57.0	57.5	58.0	58.6	59.1	59.6	60.1	60.7
120	61.2	61.7	62.3	62.8	63.3	63.9	64.4	64.9	65.5	66.0
130	66.5	67.1	67.6	68.1	68.7	69.2	69.7	70.3	70.8	71.4
140	71.9	72.4	73.0	73.5	74.0	74.6	75.1	75.7	76.2	76.7
150	77.3	77.8	78.3	78.9	79.4	80.0	80.5	81.1	81.6	82.1
160	82.7	83.2	83.8	84.3	84.8	85.4	85.9	86.5	87.0	87.6
170	88.1	88.6	89.2	89.7	90.3	90.8	91.4	91.9	92.5	93.0
180	93.6	94.1	94.7	95.2	95.7	96.3	96.8	97.4	97.9	98.5
190	99.0	99.6	100.1	100.7	101.2	101.8	102.3	102.9	103.4	104.0
200	104.5	105.1	105.7	106.2	106.8	107.3	107.9	108.4	109.0	109.5
210	110.1	110.6	111.2	111.7	112.3	112.9	113.4	114.0	114.5	115.1
220	115.6	116.2	116.8	117.3	117.9	118.4	119.0	119.6	120.1	120.7
230	121.2	121.8	122.4	122.9	123.5	124.0	124.6	125.2	125.7	126.3
240	126.8	127.4	128.0	128.5	129.1	129.7	130.2	130.8	131.4	131.9
250	132.5	133.1	133.6	134.2	134.8	135.3	135.9	136.5	137.0	137.6
260	138.2	138.7	139.3	139.9	140.4	141.0	141.6	142.2	142.7	143.3
270	143.9	144.4	145.0	145.6	146.2	146.7	147.3	147.9	148.5	149.0
280	149.6	150.2	150.8	151.3	151.9	152.5	153.1	153.6	154.2	154.8
290	155.4	155.9	156.5	157.1	157.7	158.3	158.8	159.4	160.0	160.6
300	161.2	161.7	162.3	162.9	163.5	164.1	164.7	165.2	165.8	166.4
310	167.0	167.6	168.2	168.7	169.3	169.9	170.5	171.1	171.7	172.3
320	172.8	173.4	174.0	174.6	175.2	175.8	176.4	177.0	177.5	178.1
330	178.7	179.3	179.9	180.5	181.1	181.7	182.3	182.9	183.5	184.1
340	184.6	185.2	185.8	186.4	187.0	187.6	188.2	188.8	189.4	190.0
350	190.6	191.2	191.8	192.4	193.0	193.6	194.2	194.8	195.4	196.0
360	196.6	197.2	197.8	198.4	199.0	199.6	200.2	200.8	201.4	202.0
370	202.6	203.2	203.8	204.4	205.0	205.6	206.2	206.9	207.5	208.1
380	208.7	209.3	209.9	210.5	211.1	211.7	212.3	212.9	213.5	214.2
390	214.8	215.4	216.0	216.6	217.2	217.8	218.4	219.1	219.7	220.3
400	220.9	221.5	222.1	222.7	223.4	224.0	224.6	225.2	225.8	226.4
410	227.1	227.7	228.3	228.9	229.5	230.2	230.8	231.4	232.0	232.6
420	233.3	233.9	234.5	235.1	235.8	236.4	237.0	237.6	238.3	238.9
430	239.5									

Table III.

## Lactose.

$$Cu = 0.58 + 1.25431 M \div 0.000008784 M^2.$$

Cu	0	1	2	3	4	5	6	7	8	9
0		0.3	1.1	1.9	2.7	3.5	4.3	5.1	5.9	6.7
10	7.5	8.3	9.1	9.9	10.7	11.5	12.3	13.1	13.9	14.7
20	15.5	16.3	17.1	17.9	18.7	19.5	20.3	21.1	21.9	22.7
30	23.5	24.3	25.1	25.8	26.6	27.4	28.2	29.0	29.8	30.6
40	31.4	32.2	33.0	33.8	34.6	35.4	36.2	37.0	37.8	38.6
50	39.4	40.2	41.0	41.8	42.6	43.4	44.2	45.0	45.8	46.6
60	47.4	48.2	49.0	49.8	50.6	51.4	52.2	53.0	53.8	54.6
70	55.4	56.2	57.0	57.8	58.6	59.4	60.1	60.9	61.7	62.5
80	63.3	64.1	64.9	65.7	66.5	67.3	68.1	68.9	69.7	70.5
90	71.3	72.1	72.9	73.7	74.5	75.3	76.1	76.9	77.7	78.5
100	79.3	80.1	80.9	81.7	82.5	83.3	84.1	84.9	85.7	86.5
110	87.3	88.1	88.9	89.7	90.5	91.3	92.1	92.9	93.7	94.5
120	95.3	96.1	96.9	97.6	98.4	99.2	100.0	100.8	101.6	102.4
130	103.2	104.0	104.8	105.6	106.4	107.2	108.0	108.8	109.6	110.4
140	111.2	112.0	112.8	113.6	114.4	115.2	116.0	116.8	117.6	118.4
150	119.2	120.0	120.8	121.6	122.4	123.2	124.0	124.8	125.6	126.4
160	127.2	128.0	128.8	129.6	130.4	131.2	132.0	132.8	133.6	134.4
170	135.2	136.0	136.8	137.6	138.4	139.2	140.0	140.8	141.6	142.4
180	143.2	144.0	144.8	145.6	146.4	147.2	148.0	148.8	149.6	150.4
190	151.2	152.0	152.8	153.6	154.4	155.2	156.0	156.8	157.6	158.4
200	159.2	160.0	160.8	161.6	162.4	163.2	164.0	164.8	165.6	166.4
210	167.2	168.0	168.8	169.6	170.4	171.2	172.0	172.8	173.6	174.3
220	175.1	175.9	176.7	177.5	178.3	179.1	179.9	180.7	181.5	182.3
230	183.1	183.9	184.7	185.5	186.3	187.1	187.9	188.7	189.5	190.3
240	191.1	191.9	192.7	193.5	194.3	195.1	195.9	196.7	197.5	198.3
250	199.1	199.9	200.7	201.5	202.3	203.1	203.9	204.7	205.5	206.3
260	207.1	207.9	208.7	209.5	210.3	211.1	211.9	212.7	213.5	214.3
270	215.1	215.9	216.7	217.5	218.3	219.1	219.9	220.7	221.5	222.3
280	223.1	223.9	224.7	225.5	226.3	227.1	227.9	228.7	229.5	230.3
290	231.1	231.9	232.7	233.5	234.3	235.1	235.9	236.7	237.5	238.3
300	239.1	239.9	240.7	241.5	242.3	243.1	243.9	244.7	245.5	246.3
310	247.1	247.9	248.7	249.5	250.3	251.1	251.9	252.7	253.5	254.3
320	255.1	255.9	256.7	257.5	258.3	259.1	259.9	260.7	261.5	262.3
330	263.1	263.9	264.7	265.5	266.3	267.1	267.9	268.7	269.5	270.3
340	271.1	271.9	272.7	273.5	274.3	275.1	275.9	276.7	277.5	278.3
350	279.1	279.9	280.7	281.5	282.3	283.1	283.9	284.7	285.5	286.3
360	287.1	287.9	288.7	289.5	290.3	291.1	291.9	292.7	293.5	294.3
370	295.1	295.9	296.7	297.5	298.3	299.1	299.9	300.7	301.5	302.3
380	303.1	303.9	304.7	305.5	306.3	307.1	307.9	308.7	309.5	310.3
390	311.1	311.9	312.7	313.5	314.3	315.1	315.9	316.7	317.5	318.3
400	319.1	319.9	320.8	321.6	322.4	323.2	324.0	324.8	325.6	326.4
410	327.2	328.0	328.8	329.6	330.4	331.2	332.0	332.8	333.6	334.4
420	335.2	336.0	336.8	337.6	338.4	339.2	340.0	340.8	341.6	342.4
430	343.2	344.0	344.8	345.6	346.4	347.2	348.0	348.8	349.6	350.4
440	351.2	352.0	352.8	353.6	354.4	355.2				

Table IV.

0.200 gr. Saccharose + Lactose.

Cu = 1.94 + 1.2597 M ÷ 0.00000025 M<sup>2</sup>.

Cu	0	1	2	3	4	5	6	7	8	9
0					1.6	2.4	3.2	4.0	4.8	5.6
10	6.4	7.2	8.0	8.8	9.6	10.4	11.2	12.0	12.8	13.5
20	14.3	15.1	15.9	16.7	17.5	18.3	19.1	19.9	20.7	21.5
30	22.3	23.1	23.9	24.7	25.5	26.2	27.0	27.8	28.6	29.4
40	30.2	31.0	31.8	32.6	33.4	34.2	35.0	35.8	36.7	37.4
50	38.2	38.9	39.7	40.5	41.3	42.1	42.9	43.7	44.5	45.3
60	46.1	46.9	47.7	48.5	49.3	50.1	50.9	51.7	52.4	53.2
70	54.0	54.8	55.6	56.4	57.2	58.0	58.8	59.6	60.4	61.2
80	62.0	62.8	63.6	64.4	65.1	65.9	66.7	67.5	68.3	69.1
90	69.9	70.7	71.5	72.3	73.1	73.9	74.7	75.5	76.3	77.1
100	77.9	78.6	79.4	80.2	81.0	81.8	82.6	83.4	84.2	85.0
110	85.8	86.6	87.4	88.2	89.0	89.8	90.6	91.3	92.1	92.9
120	93.7	94.5	95.3	96.1	96.9	97.7	98.5	99.3	100.1	100.9
130	101.7	102.5	103.3	104.0	104.8	105.6	106.4	107.2	108.0	108.8
140	109.6	110.4	111.2	112.0	112.8	113.6	114.4	115.2	116.0	116.8
150	117.5	118.3	119.1	119.9	120.7	121.5	122.3	123.1	123.9	124.7
160	125.5	126.3	127.1	127.9	128.7	129.5	130.2	131.0	131.8	132.6
170	133.4	134.2	135.0	135.8	136.6	137.4	138.2	139.0	139.8	140.6
180	141.4	142.2	142.9	143.7	144.5	145.3	146.1	146.9	147.7	148.5
190	149.3	150.1	150.9	151.7	152.5	153.3	154.1	154.9	155.7	156.4
200	157.2	158.0	158.8	159.6	160.4	161.2	162.0	162.8	163.6	164.4
210	165.2	166.0	166.8	167.6	168.4	169.1	169.9	170.7	171.5	172.3
220	173.1	173.9	174.7	175.5	176.3	177.1	177.9	178.7	179.5	180.3
230	181.1	181.9	182.6	183.4	184.2	185.0	185.8	186.6	187.4	188.2
240	189.0	189.8	190.6	191.4	192.2	193.0	193.8	194.6	195.3	196.1
250	196.9	197.7	198.5	199.3	200.1	200.9	201.7	202.5	203.3	204.1
260	204.9	205.7	206.5	207.3	208.0	208.8	209.6	210.4	211.2	212.0
270	212.8	213.6	214.4	215.2	216.0	216.8	217.6	218.4	219.2	220.0
280	220.8	221.5	222.3	223.1	223.9	224.7	225.5	226.3	227.1	227.9
290	228.7	229.5	230.3	231.1	231.9	232.7	233.5	234.2	235.0	235.8
300	236.6	237.4	238.2	239.0	239.8	240.6	241.4	242.2	243.0	243.8
310	244.6	245.4	246.2	247.0	247.7	248.5	249.3	250.1	250.9	251.7
320	252.5	253.3	254.1	254.9	255.7	256.5	257.3	258.1	258.9	259.7
330	260.4	261.2	262.0	262.8	263.6	264.4	265.2	266.0	266.8	267.6
340	268.4	269.2	270.0	270.8	271.6	272.4	273.1	273.9	274.7	275.5
350	276.3	277.1	277.9	278.7	279.5	280.3	281.1	281.9	282.7	283.5
360	284.3	285.1	285.9	286.6	287.4	288.2	289.0	289.8	290.6	291.4
370	292.2	293.0	293.8	294.6	295.4	296.2	297.0	297.8	298.6	299.3
380	300.1	300.9	301.7	302.5	303.3	304.1	304.9	305.7	306.5	307.3
390	308.1	308.9	309.7	310.5	311.3	312.1	312.8	313.6	314.4	315.2
400	316.0	316.8	317.6	318.4	319.2	320.0	320.8	321.6	322.4	323.2
410	324.0	324.8	325.5	326.3	327.1	327.9	328.7	329.5	330.3	331.1
420	331.9	332.7	333.5	334.3	335.1	335.9	336.7	337.5	338.2	339.0
430	339.8	340.6	341.4	342.2	343.0	343.8	344.6	345.4	346.2	347.0
440	347.8	348.6	349.4	350.2	351.0	351.7				



Table V.

50 mgr. Lactose + Sucre interverti.

$$Cu = 64.91 + 1.897912 \text{ l} \div 0.00050226 \text{ l}^2.$$

Cu	0	1	2	3	4	5	6	7	8	9
70	2.7	3.2	3.7	4.3	4.8	5.3	5.9	6.4	6.9	7.4
80	8.0	8.5	9.0	9.6	10.1	10.6	11.1	11.7	12.2	12.7
90	13.3	13.8	14.3	14.9	15.4	15.9	16.5	17.0	17.5	18.0
100	18.6	19.1	19.6	20.2	20.7	21.2	21.8	22.3	22.8	23.4
110	23.9	24.4	25.0	25.5	26.0	26.6	27.1	27.7	28.2	28.7
120	29.3	29.8	30.3	30.9	31.4	31.9	32.5	33.0	33.5	34.1
130	34.6	35.2	35.7	36.2	36.8	37.3	37.8	38.4	38.9	39.5
140	40.0	40.5	41.1	41.6	42.1	42.7	43.2	43.8	44.3	44.8
150	45.4	45.9	46.5	47.0	47.5	48.1	48.6	49.2	49.7	50.2
160	50.8	51.3	51.9	52.4	53.0	53.5	54.0	54.6	55.1	55.7
170	56.2	56.8	57.3	57.8	58.4	58.9	59.5	60.0	60.6	61.1
180	61.6	62.2	62.7	63.3	63.8	64.4	64.9	65.5	66.0	66.6
190	67.1	67.6	68.2	68.7	69.3	69.8	70.4	70.9	71.5	72.0
200	72.6	73.1	73.7	74.2	74.8	75.3	75.9	76.4	77.0	77.5
210	78.1	78.6	79.2	79.7	80.3	80.8	81.4	81.9	82.5	83.0
220	83.6	84.1	84.7	85.2	85.8	86.3	86.9	87.4	88.0	88.5
230	89.1	89.6	90.2	90.7	91.3	91.9	92.4	93.0	93.5	94.1
240	94.6	95.2	95.7	96.3	96.8	97.4	98.0	98.5	99.1	99.6
250	100.2	100.7	101.3	101.9	102.4	103.0	103.5	104.1	104.6	105.2
260	105.8	106.3	106.9	107.4	108.0	108.5	109.1	109.7	110.2	110.8
270	111.3	111.9	112.5	113.0	113.6	114.1	114.7	115.3	115.8	116.4
280	117.0	117.5	118.1	118.6	119.2	119.8	120.3	120.9	121.4	122.0
290	122.6	123.1	123.7	124.3	124.8	125.4	126.0	126.5	127.1	127.7
300	128.2	128.8	129.4	129.9	130.5	131.1	131.6	132.2	132.7	133.3
310	133.9	134.4	135.0	135.6	136.2	136.7	137.3	137.9	138.4	139.0
320	139.6	140.1	140.7	141.3	141.8	142.4	143.0	143.5	144.1	144.7
330	145.3	145.8	146.4	147.0	147.5	148.1	148.7	149.3	149.8	150.4
340	151.0	151.5	152.1	152.7	153.3	153.8	154.4	155.0	155.6	156.1
350	156.7	157.3	157.9	158.4	159.0	159.6	160.2	160.7	161.3	161.9
360	162.5	163.0	163.6	164.2	164.8	165.4	165.9	166.5	167.1	167.7
370	168.2	168.8	169.4	170.0	170.6	171.1	171.7	172.3	172.9	173.5
380	174.0	174.6	175.2	175.8	176.4	176.9	177.5	178.1	178.7	179.3
390	179.8	180.4	181.0	181.6	182.2	182.8	183.3	183.9	184.5	185.1
400	185.7	186.3	186.9	187.4	188.0	188.6	189.2	189.8	190.4	190.9
410	191.5	192.1	192.7	193.3	193.9	194.5	195.1	195.6	196.2	196.8
420	197.4	198.0	198.6	199.2	199.8	200.4	200.9	201.6	202.1	202.7
430	203.3	203.9	204.5	205.1	205.7	206.3	206.9	207.4	208.0	208.6
440	209.2	209.8	210.4	211.0	211.6	212.2				

En avril 1923.



COMPTES-RENDUS

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---

15<sup>ME</sup> VOLUME.

No. 4



COPENHAGUE

EN COMMISSION CHEZ H. HAGERUP

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1924

*Prix: 2 Kr. 50 Øre*



# ON THE SALTING-OUT EFFECT.

BY

K. LINDERSTRØM-LANG.

## Introduction.

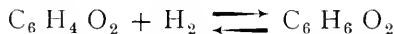
The influence of salts on the solubility of neutral substances has been investigated by many writers. The ratio between the solubility  $s_0$  in water and  $s_n$  in a salt solution of the concentration  $c_n$  is a measure of this influence, and from the numerous investigations made, it appears that equations of the form

$$\log s_0/s_n = k \cdot c_n \quad (1)$$

or 
$$s_0/s_n - 1 = k^1 \cdot c_n \quad (2)$$

seem approximately to describe the phenomena in question.  $k$  (or  $k^1$  as the case may be) is a constant, independent of  $c_n$  but varying with the temperature and with the nature both of the salt and the neutral substance.

The present work is an attempt to arrive at some of the causes of this variation. The experiments were carried out in the Chemical Department of the Carlsberg Laboratory, as a continuation of the work on the Biilmann quinhydrone electrode published by S. P. L. Sørensen, Margrethe Sørensen and K. Linderstrøm-Lang in 1921. It was here shown how the potential of the quinhydrone electrode must vary with the salt concentration of the liquid whose concentration of hydrogen ions was to be measured, an alteration taking place in the equilibrium hydrogen pressure in the process:



The cause of this alteration was found to be the difference in the influence of the salt on the »active mass«, or the activity of the two components of the quinhydrone, quinone and hydroquinone, and to be closely related to be phenomena above mentioned.

It will, I think, not be out of place here to set forth briefly the formal treatment of the activity question, as developed by Lewis, Bjerrum and Brønsted.

The differential work of solution for one gram-molecule of the neutral substance S in its diluted watery solution of the concentration  $s_0$  (mole per litre), where the gas laws are presumed to hold good, may be set as:

$$A_0 = RT \ln s_0 + i. \quad (3)$$

Through the work of solution A for S in any other solvent, e. g. a salt solution or a solution of another substance at concentration  $c_n$  — or in a solution of S at so high a concentration that the gas laws would not hold good — we can now define a quantity, the activity of S,  $a_s$ , where:

$$A = RT \ln a_s + i \quad (4)$$

must apply exactly.

The ratio between  $a_s$  and  $s$  (concentration of S) is called the activity coefficient, and is written  $f$ ; i. e.

$$\frac{a_s}{s} = f. \quad (5)$$

$$A = RT \ln sf + i.$$

If S is present in a high concentration  $s$ , in a non-saline solution,  $f$  is a function of  $s$  alone, and becomes 1 when  $s$  approaches 0 ( $s_0$ ). If, on the other hand, there is salt (or any other substance) present, then the matter will be simplified by choosing  $s$  so low that the gas laws for S hold good in each separate salt solution, as  $f$  will then be a function of  $c_n$  alone, and approaches 1 when  $c_n$  approaches 0.

Taking  $s = s_1$  in water, and  $s = s_n$  in salt solution, so that the solution is saturated with S in both cases, then

$$A_1 = A_n = 0. \quad RT \ln s_1 f_1 = RT \ln s_n f_n, \text{ whence } \frac{s_1}{s_n} = \frac{f_n}{f_1}, \text{ which, when S is slightly soluble, becomes}$$

$$\frac{s_0}{s_n} = f, \quad (6)$$

where  $f$  again is a function of  $c_n$  alone. We can thus determine  $f$  by measuring the solubility of S in the respective solutions, as a function of  $c_n$ , as (1) and (2) indicate.

If S be found at the same slight concentration in two salt solutions with the concentrations  $c$  and  $c + dc$ , then the work of transference is

$$dA = RT \ln a_s = RT \frac{d \ln f}{dc} dc. \quad (7)$$

If S be found in two watery solutions at concentrations  $s$  and  $s + ds$ , where  $s$  is assumed to be high, we then get the work of transference

$$dA = RT \ln a_s = RT \ln s + RT \frac{d \ln f}{ds} ds. \quad (8)$$

Owing to the thermodynamic contents of the law of mass-action, by a reciprocal process:

$S \rightleftharpoons S' + S''$ ,  $f$  enters into the mass action equation, as  $K$  must be a constant in the equation:

$$\frac{a'_s \cdot a''_s}{a_s} = K, \quad \frac{f' \cdot f''}{f} = K \cdot \frac{s}{s' \cdot s''}.$$

In the process of the quinhydrone electrode, therefore, the hydrogen pressure, or hydrogen activity, whichever we may call it, will alter with the salt concentration, as

$$a_{H_2} = \frac{a_{\text{Hydroq.}}}{a_{\text{Quinon.}}} \cdot K = \frac{f_{\text{Hydroq.}}}{f_{\text{Quinon.}}} \cdot K,$$

as  $s_{\text{Hydroquinone}} = s_{\text{Quinone}}$ .

Physically speaking,  $f$  means that  $S$  is in another state in its concentrated solution, or in a salt solution, than it is in pure water. Other forces act on it; it is associated, it forms chemical compounds, or is decomposed from such.

When, therefore, on adding salt, the hydrogen pressure of the quinhydrone electrode alters, when the solubility of the two components is altered, we must look for the cause of this in the forces with which the ions act on the molecules of these substances. It would therefore be of considerable interest to extend the earlier investigations on these substances which only embraced solutions of sodium chloride, to other salts as well, both theoretically for elucidation of conditions in aqueous solutions and practically for the purpose of measuring with the Biilmann electrode in its first form, which offers great advantages in point of facility and accuracy.

I have therefore, by determinations of solubility, found  $f$  for hydroquinone and quinone in solutions of the following salts:

Anions:

Monovalent....  $\text{Cl}^- \text{Br}^- \text{J}^-$   
 Divalent.....  $\text{SO}_4^{--}$

Kations:

Monovalent....  $\text{H}^+ \text{Li}^+ \text{Na}^+ \text{K}^+ \text{Rb}^+ \text{Cs}^+$   
 Divalent.....  $\text{Mg}^{++} \text{Ca}^{++} \text{Sr}^{++} \text{Ba}^{++}$   
 Trivalent.....  $\text{Al}^{+++} \text{La}^{+++}$

I have also, in the case of some of these salts, determined  $f$  for boracic and succinic acid in the same way. I include these investigations here, as they were in some degree of use in the theoretical treatment of the conditions for hydroquinone and quinone.

It is my very pleasant duty to thank the Leader of the Chemical Department at the Carlsberg Laboratory, Professor S. P. L. Sorensen, for the manner in which he has facilitated my work and for valuable advice during the past years — and to thank Prof. N. Bjerrum for very kindly reading through my work and discussing it with me.

I also wish to thank Docent J. A. Christiansen for advice and criticism, and cand. polyt. Holger Jørgensen for many instructive talks.

## A. EXPERIMENTAL SECTION.

### 1. Solubility of Hydroquinone.

The determination of solubility was carried out in the following manner: Most of the experiments were made at  $18^\circ$ , but a large number also at  $24^\circ$ , which rendered it possible to obtain an approximate value for the alteration in heat of solution with the concentration of salt. In each series of experiments, which as a rule comprised three salts, I have used 4—5 different concentrations for each salt, ranging from 0.4—0.8 up to 3—5 normal. At the same time, a separate determination of solubility was always made in 0.01  $n$  hydrochloric acid, often a double determination. This increased the accuracy in each series of experiments, and thus also the accuracy in determination of  $f$ . The hydrochloric acid concentration 0.01  $n$  I have used throughout all the experiments, and not taken into account as salt concentration.  $s_0/s_n$  ( $= 10^{k \cdot c_n}$  according to (1)) is thus the ratio between solubility of hydroquinone in 0.01  $n$  HCl and in a solution of salt at concentration  $c_n$  in 0.01  $n$  HCl. We thus avoid the somewhat uncertain extrapolation to pure water. The hydrochloric acid is necessary owing to the instability of the hydroquinone at lower concentrations of hydrogen ions.

As regards the purity of the preparations employed, the hydroquinone here used was the trade article once recrystallised. Various preparations made at different times and from different trade products showed the same solubility. During the recrystallisation, carbondioxyde was passed through the solution of



hydroquinone, this rendering the preparation a fine white, though with a faint blueish-violet tinge.

As to salts, Kahlbaums »Zur Analyse« were used, as a rule without recrystallisation, as slight impurities would only have a very slight effect on the solubility. Special tests for purity were only made in the case of the lithium chloride, which was examined by treating with amyl alcohol and found to be pure. Of RbCl, CsCl and LaCl<sub>3</sub>, the available quantities were so small that purity tests could not be made. In the case of RbCl and CsCl however, I have made parallel experiments with two different preparations (among them an English one, lent me by Prof. Bronsted, of the University physio-chemical Laboratory, for which I beg to express my thanks) and found them to agree.

From these salts, a stock solution was prepared, as strong as possible, and filtered. Normality was determined by chlorine titration in the case of the chlorides, or by evaporation and weighing (sulphates). From this stock solution, the experimental liquids were prepared by dilution in a measuring flask.

The actual process of experiment was as follows:

Some 25—50 cc of the salt solution in which the solubility was to be measured was placed in 100 cc medicine bottles and shaken in a thermostate with a suitable, weighed, quantity of hydroquinone. The shaking was carried on for 3—5 hours, the temperature during that time not varying more than 0°.02. By taking samples at different times it was found that the period in question was sufficient to produce saturation. When the shaking was finished, the mother liquor was drawn out through a stopper of cottonwool, and suitable quantities drawn off with standardized pipettes. The titration was carried out according to Valeur<sup>1)</sup> with iodine in bicarbonate alkaline solution, the retitration with sodium thiosulphate, using starch as indicator. Further as to this method, see "Sur l'erreur de sel inhérente à l'électrode de quinhydrone" the work referred to on p. 1.<sup>2)3)</sup>.

In the following tables, the results will be found. The solubility *s* is indicated in gram-molecules per litre, the salt concentration in gram-equivalents per litre. *k* is calculated according to (1).

$$k = \frac{\log f}{c}.$$

<sup>1)</sup> Ann. de Chim. et de Phys. (7) **21**, 528 (1900).

<sup>2)</sup> Comptes-rendus du Lab. Carlsberg **14**, Nr. 14 (1921).

<sup>3)</sup> Ann. de Chim. (9) **16** (1921).

	c	s	f	log f	k
Table 1a. LiCl. $18^{\circ} \pm 0^{\circ}.02$ .					
0.01 n HCl + o		0.5103	1.000	0.0000	—
—	0.835	0.3747	1.362	0.1342	0.161
—	2.087	0.2362	2.160	0.3345	0.160
—	3.340	0.1493	3.418	0.5338	0.160
—	4.175	0.1117	4.568	0.6597	0.158
—	6.262	0.0577	8.844	0.9467	(0.151)

Table 1 b. LiCl. $23^{\circ}.75$ .					
0.01 n HCl + o		0.6180	1.000	0.0000	—
—	0.669	0.4844	1.276	0.1059	0.158
—	1.338	0.3787	1.632	0.2127	0.159
—	2.676	0.2324	2.659	0.4247	0.159
—	4.113	0.1427	4.331	0.6366	0.155

Table 2a. NaCl. $18^{\circ}$ .					
0.01 n HCl + o		0.5103	1.000	0.0000	—
—	0.49	0.4270	1.195	0.0774	0.158
—	0.99	0.3579	1.426	0.1541	0.156
—	1.99	0.2496	2.044	0.3105	0.156
—	2.99	0.1752	2.913	0.4643	0.155
—	3.99	0.1199	4.256	0.6290	0.158

Table 2 b. NaCl. $23^{\circ}.75$ .					
0.01 n HCl + o		0.6180	1.000	0.0000	—
—	0.4	0.5353	1.155	0.0626	0.157
—	0.8	0.4631	1.335	0.1255	0.157
—	1.6	0.3487	1.772	0.2485	0.155
—	3.2	0.1950	3.169	0.5009	0.157

Table 3 a. KCl. $18^{\circ}$ .					
0.01 n HCl + o		0.5103	1.000	0.0000	—
—	0.7	0.4258	1.198	0.0785	0.112
—	1.4	0.3530	1.446	0.1602	0.114
—	2.1	0.2940	1.736	0.2396	0.114
—	2.8	0.2546	2.004	0.3019	0.108

Table 3 b. KCl. $23^{\circ}.75$ .					
0.01 n HCl + o		0.6180	1.000	0.0000	—
—	0.7	0.5132	1.204	0.0806	0.115
—	1.4	0.4278	1.445	0.1599	0.114
—	2.8	0.3010	2.053	0.3124	0.112

	c	s	f	log f	k
Table 4. RbCl. $18^{\circ} \pm 0^{\circ}.02$ .					
0.01 n HCl + 0		0.5103	1.000	0.0000	—
—	1.2	0.4075	1.252	0.0976	0.081
—	1.8	0.3665	1.392	0.1433	0.080
—	2.4	0.3413	1.495	0.1746	0.073

Table 5 a. CsCl. $18^{\circ}$ .					
0.01 n HCl + 0		0.5103	1.000	0.0000	—
—	0.578	0.4983	1.024	0.0103	0.018
—	0.867	0.4961	1.029	0.0124	0.014
—	1.733	0.4913	1.039	0.0166	0.010

Table 5 b. CsCl. $23^{\circ}.75$ .					
0.01 n HCl + 0		0.6180	1.000	0.0000	—
—	0.479	0.6045	1.022	0.0094	0.020
—	0.959	0.5964	1.036	0.0153	0.016
—	1.438	0.5890	1.049	0.0208	0.014

Table 6. KBr. $18^{\circ}$ .					
0.01 n HCl + 0		0.5099	1.000	0.0000	—
—	0.6	0.4394	1.160	0.0645	0.108
—	1.2	0.3787	1.347	0.1294	0.108
—	1.8	0.3281	1.554	0.1915	0.106

Table 7. KJ. $18^{\circ}$ .					
0.01 n HCl + 0		0.5103	1.000	0.0000	—
—	0.6	0.4427	1.153	0.0618	0.103
—	1.2	0.3923	1.301	0.1143	0.095
—	1.8	0.3457	1.476	0.1691	0.094
—	2.4	0.3045	1.676	0.2243	0.093
—	2.82	0.2791	1.828	0.2620	0.093

Table 8. MgCl <sub>2</sub> . $18^{\circ}$ .					
0.01 n HCl + 0		0.5103	1.000	0.0000	—
—	0.495	0.4299	1.187	0.0745	0.151
—	0.989	0.3635	1.404	0.1474	0.149
—	1.979	0.2589	1.971	0.2947	0.149
—	3.958	0.1308	3.901	0.5912	0.149

	c	s	f	log f	k
Table 9 a. $\text{CaCl}_2$ . $18^\circ \pm 0^\circ.02$ .					
0.01 n HCl + o		0.5103	1.000	0.0000	—
—	0.598	0.4180	1.221	0.0867	0.145
—	1.195	0.3408	1.497	0.1752	0.147
—	2.390	0.2263	2.255	0.3532	0.148
—	4.780	0.0978	5.218	0.7175	0.150

Table 9 b. $\text{CaCl}_2$ . $23^\circ.75$ .					
0.01 n HCl + o		0.6180	1.000	0.0000	—
—	0.598	0.5072	1.218	0.0857	0.143
—	1.195	0.4177	1.480	0.1703	0.143
—	2.390	0.2786	2.218	0.3460	0.145
—	3.585	0.1850	3.341	0.5239	0.146

Table 10. $\text{SrCl}_2$ . $18^\circ$ .					
0.01 n HCl + o		0.5103	1.000	0.0000	—
—	0.4	0.4444	1.148	0.0599	0.150
—	0.8	0.3894	1.310	0.1173	0.147
—	1.6	0.2948	1.731	0.2383	0.149
—	3.2	0.1636	3.119	0.4940	0.154

Table 11 a. $\text{BaCl}_2$ . $18^\circ$ .					
0.01 n HCl + o		0.5103	1.000	0.0000	—
—	0.4	0.4449	1.147	0.0596	0.149
—	1.2	0.3417	1.493	0.1741	0.145
—	1.5	0.3068	1.663	0.2209	0.147
—	1.6	0.2983	1.711	0.2333	0.146
—	2.0	0.2606	1.958	0.2918	0.146

Table 11 b. $\text{BaCl}_2$ . $23^\circ.75$ .					
0.01 n HCl + o		0.6180	1.000	0.0000	—
—	0.4	0.5385	1.148	0.0599	0.150
—	0.8	0.4724	1.308	0.1166	0.146
—	1.2	0.4139	1.493	0.1741	0.145
—	1.6	0.3606	1.714	0.2340	0.146

Table 12. $\text{AlCl}_3$ . $18^\circ$ .					
0.01 n HCl + o		0.5103	1.000	0.0000	—
—	0.477	0.4273	1.194	0.0770	0.161
—	1.192	0.3291	1.551	0.1906	0.160
—	2.385	0.2099	2.431	0.3858	0.162
—	4.770	0.0815	6.261	0.7966	0.167

	c	s	f	log f	k
Table 13. $\text{LaCl}_3$ . $18^\circ \pm 0.02$ .					
0.01 n HCl + o		0.5103	1.000	0.0000	—
—	0.497	0.4360	1.170	0.0682	0.137
—	1.242	0.3478	1.467	0.1664	0.134
—	2.485	0.2349	2.172	0.3369	0.136
—	4.970	0.1053	4.846	0.6854	0.138

Table 14. $\text{MgSO}_4$ . $18^\circ$ .					
0.01 n HCl + o		0.5088	1.000	0.0000	—
—	0.978	0.3708	1.372	0.1374	0.140
—	1.956	0.2632	1.933	0.2862	0.146
—	2.934	0.1809	2.813	0.4492	0.153

Table 15. $18^\circ$ .					
HCl	0.01	0.5103	1.000	0.0000	—
$\text{H}_2\text{SO}_4$	1.5	0.3760	1.357	0.1326	0.089
HCl	1.5	0.3520	1.450	0.1614	0.108

Table 16. $\text{Na}_2\text{SO}_4$ . $18^\circ$ .					
0.01 n HCl + o		0.5103	1.000	0.0000	—
—	1.5	0.2976	1.715	0.2343	0.156

I have also included the effect of a neutral substance, mannite, on the solubility of the hydroquinone.

Table 17. Mannit. $18^\circ \pm 0.02$ .					
	c (Mol)	s	f	log f	k
0.01 n HCl + o		0.5103	1.000	0.0000	—
—	0.2	0.5030	1.015	0.0065	0.033
—	0.4	0.4925	1.036	0.0154	0.039
—	0.6	0.4828	1.057	0.0241	0.040
—	0.8	0.4721	1.081	0.0338	0.042

The values for  $s_0$

0.5103	$18^\circ$
0.6180	$23^\circ.75$

were found as mean values between the values from the separate series of experiments, where the highest divergence was 5 ‰. Only in the case of KBr and  $\text{MgSO}_4$ , where the determination was made at a different time, have I retained the separate value. I estimate the accuracy of f at 3—4 ‰.

Figs. 1, 2 and 3 show the variation of  $f_{18}^{\circ}$  with the salt concentration,  $\log f$  being the abscissa and the salt normality the ordinate.

Fig. 1.

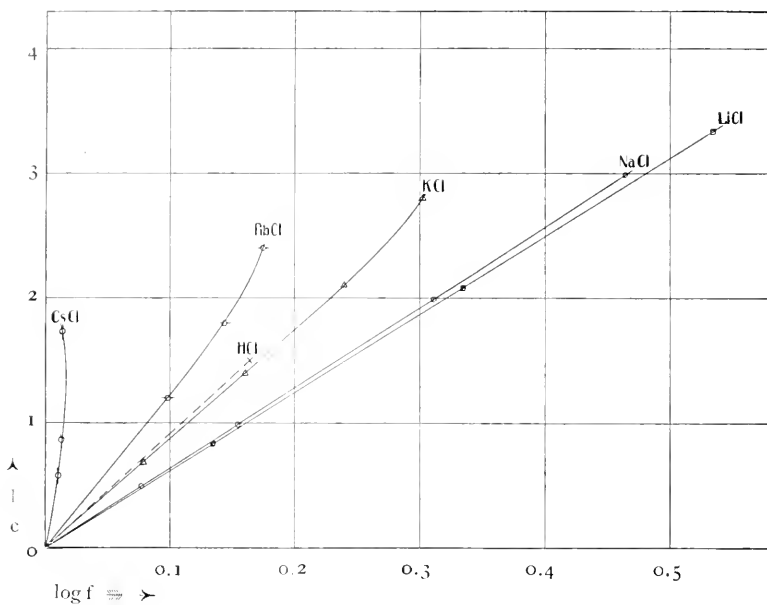


Fig. 2.

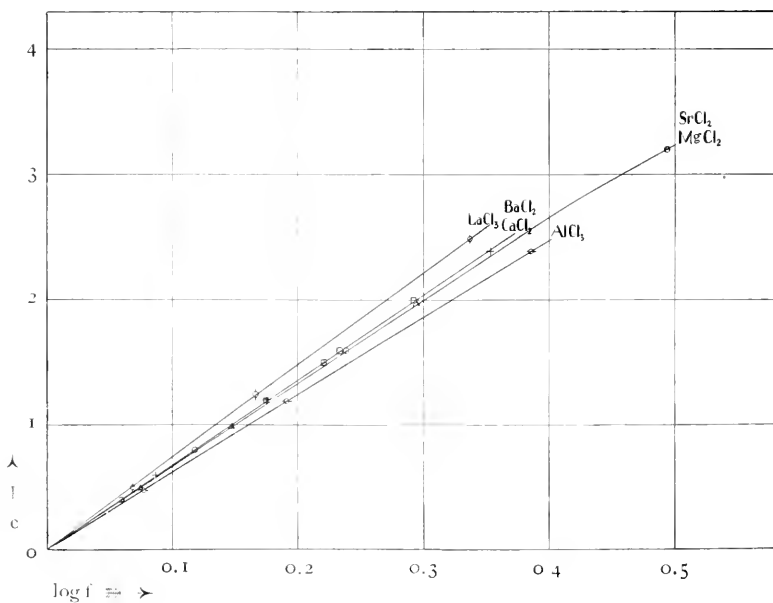
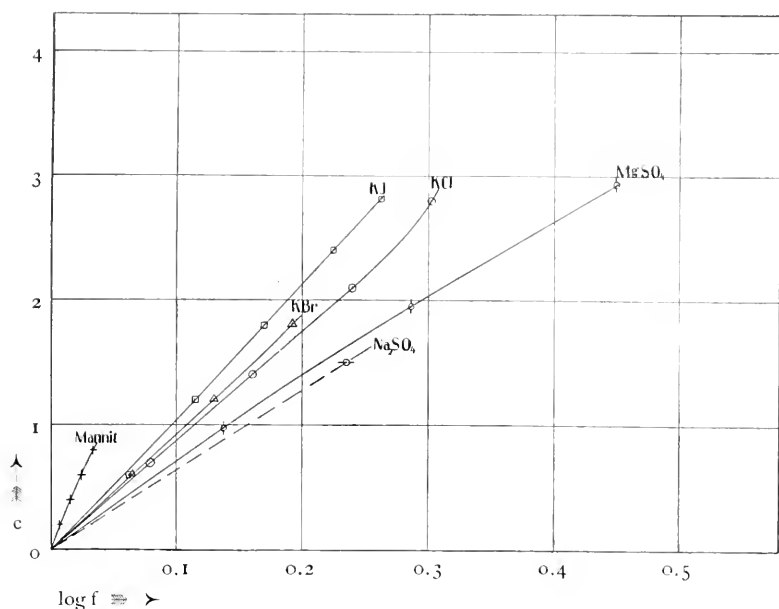


Fig. 3.



## 2. Solubility of Quinone.

The quinone was prepared by distilling a raw quinone with water vapour (see also "Sur l'erreur de sel inhérente à l'électrode de quinhydrone"). The temperatures used were abt. 12°, abt. 24° and 18°. The titration was made according to Knecht and Hibbert<sup>1)</sup> with titanium chloride in acid solution, retitration with iron alum, using potassium rhodanide as indicator. Otherwise, as with hydroquinone.

Table 18a. LiCl. 11°.85 ± 0°.02.

	c	s	f	log f	k
0.01 n HCl + 0		0.0863	1.000	0.0000	—
—	0.835	0.0744	1.159	0.0641	0.077
—	1.670	0.0661	1.305	0.1156	0.069
—	2.505	0.0604	1.429	0.1550	0.062
—	3.340	0.0558	1.546	0.1892	0.057

<sup>1)</sup> Ber. deutsch. chem. Gesellsch. **43**, 3455 (1910).

	c	s	f	log f	k
Table 18b. LiCl. $18^{\circ} \pm 0^{\circ}.02$ .					
0.01 n HCl + 0		0.1030	1.000	0.0000	—
—	0.669	0.0908	1.134	0.0546	0.082
—	1.338	0.0818	1.259	0.1000	0.075
—	2.676	0.0692	1.488	0.1726	0.066
—	4.013	0.0625	1.648	0.2170	0.054
—	5.351	0.0593	1.737	0.2398	0.040

Table 18c. LiCl. $23^{\circ}.85$ .					
0.01 n HCl + 0		0.1233	1.000	0.0000	—
—	0.835	0.1045	1.180	0.0719	0.086
—	1.670	0.0922	1.336	0.1258	0.075
—	2.505	0.0830	1.484	0.1714	0.068
—	3.340	0.0760	1.623	0.2103	0.063

Table 19a. NaCl. $11^{\circ}.85$ .					
0.01 n HCl + 0		0.0863	1.000	0.0000	—
—	0.4	0.0816	1.057	0.0241	0.060
—	0.8	0.0781	1.105	0.0434	0.054
—	1.6	0.0718	1.202	0.0799	0.050
—	3.2	0.0620	1.392	0.1436	0.045

Table 19b. NaCl. $18^{\circ}$ .					
0.01 n HCl + 0		0.1037	1.000	0.0000	—
—	0.49	0.0961	1.079	0.0330	0.067
—	0.99	0.0905	1.146	0.0592	0.060
—	1.99	0.0804	1.290	0.1106	0.056
—	2.99	0.0726	1.428	0.1547	0.052
—	3.99	0.0661	1.569	0.1956	0.049

Table 19c. NaCl. $23^{\circ}.85$ .					
0.01 n HCl + 0		0.1233	1.000	0.0000	—
—	0.4	0.1152	1.070	0.0294	0.074
—	0.8	0.1092	1.129	0.0527	0.066
—	1.6	0.0974	1.266	0.1024	0.064
—	3.2	0.0821	1.502	0.1770	0.055

Table 20a. KCl. $11^{\circ}.85$ .					
0.01 n HCl + 0		0.0863	1.000	0.0000	—
—	0.7	0.0839	1.028	0.0120	0.017
—	1.4	0.0826	1.045	0.0191	0.014
—	2.1	0.0808	1.068	0.0286	0.014
—	2.8	0.0792	1.090	0.0374	0.013



	c	s	f	log f	k
Table 20b. KCl. $18^{\circ} \pm 0^{\circ}.02$ .					
0.01 n H Cl + 0		0.1031	1.000	0.0000	—
—	0.7	0.0987	1.045	0.0191	0.027
—	1.4	0.0956	1.078	0.0326	0.023
—	2.1	0.0928	1.111	0.0457	0.022
—	2.8	0.0894	1.153	0.0618	0.022

Table 20c. KCl. $23^{\circ}.85$ .					
0.01 n H Cl + 0		0.1233	1.000	0.0000	—
—	0.7	0.1167	1.057	0.0241	0.034
—	1.4	0.1119	1.101	0.0418	0.030
—	2.1	0.1073	1.149	0.0603	0.029
—	2.8	0.1026	1.201	0.0795	0.028

Table 21. RbCl. $18^{\circ}$					
0.01 n H Cl + 0		0.1033	1.000	0.0000	—
—	0.6	0.1006	1.027	0.0116	0.019
—	1.2	0.0979	1.055	0.0233	0.019
—	2.4	0.0941	1.098	0.0406	0.017

Table 22. CsCl. $18^{\circ}$ .					
0.01 n H Cl + 0		0.1036	1.000	0.0000	—
—	0.578	0.1016	1.020	0.0086	0.015
—	0.867	0.1019	1.017	0.0073	0.008
—	1.733	0.1002	1.034	0.0145	0.008

Table 23a. KBr. $17^{\circ}.85$ .					
0.01 n H Cl + 0		0.1028	1.000	0.0000	—
—	0.6	0.1086	0.947	—0.0237	—0.040
—	1.2	0.1152	0.892	—0.0494	—0.041
—	1.95	0.1227	0.838	—0.0769	—0.039

Table 23b. KBr. $23^{\circ}.75$ .					
0.01 n H Cl + 0		0.1240	1.000	0.0000	—
—	0.6	0.1277	0.971	—0.0130	—0.022
—	1.2	0.1342	0.924	—0.0345	—0.029
—	1.95	0.1408	0.880	—0.0553	—0.028

Table 24a. CaCl <sub>2</sub> . $17^{\circ}.85$ .					
0.01 n H Cl + 0		0.1028	1.000	0.0000	—
—	0.598	0.0938	1.095	0.0394	0.066
—	1.195	0.0872	1.179	0.0715	0.060
—	2.390	0.0777	1.324	0.1219	0.051

	c	s	f	log f	k
Table 24 b. $\text{CaCl}_2$ . $23^\circ.75 \pm 0^\circ.02$ .					
0.01 n HCl + o		0.1238	1.000	0.0000	—
—	0.598	0.1112	1.123	0.0504	0.084
—	1.195	0.1026	1.207	0.0817	0.068
—	2.390	0.0903	1.371	0.1370	0.057
—	3.585	0.0819	1.512	0.1796	0.050

Table 25 a. $\text{Na}_2\text{SO}_4$ . $17^\circ.85$ .					
0.01 n HCl + o		0.1028	1.000	0.0000	—
—	0.36	0.0876	1.174	0.0697	0.194
—	0.72	0.0754	1.364	0.1348	0.187
—	1.44	0.0551	1.865	0.2707	0.188

Table 25 b. $\text{Na}_2\text{SO}_4$ . $23^\circ.75$ .					
0.01 n HCl + o		0.1240	1.000	0.0000	—
—	0.36	0.1041	1.191	0.0759	0.211
—	0.72	0.0897	1.383	0.1408	0.196
—	1.44	0.0657	1.888	0.2760	0.192

Table 26. $\text{MgSO}_4$ . $18^\circ$ .					
0.01 n HCl + o		0.1040	1.000	0.0000	—
—	1.0	0.0693	1.501	0.1764	0.176
—	2.0	0.0458	2.271	0.3562	0.178
—	3.0	0.0294	3.537	0.5486	0.183

Table 27. $18^\circ$ .					
0.01 n HCl + o		0.1037	1.000	0.0000	—
—	1.5 n $\text{BaCl}_2$	0.0883	1.174	0.0697	0.046
—	1.49 n HCl	0.1139	0.910	—0.0410	—0.027
—	1.49 n $\text{H}_2\text{SO}_4$	0.0944	1.099	0.0410	0.027

From all measurements for solubility in 0.01 n HCl we obtain the following values for  $s_0$ :

0.0863  $11^\circ.85$

0.1033  $18^\circ$

0.1236  $23^\circ.80$

The accuracy from one series of experiments to another is abt. 7 ‰, within each series separately 3—4 ‰.

As in the case of the hydroquinone, the relation between  $f_{18^\circ}$  and c is shown graphically in Figs. 4 and 5.

Fig. 4.

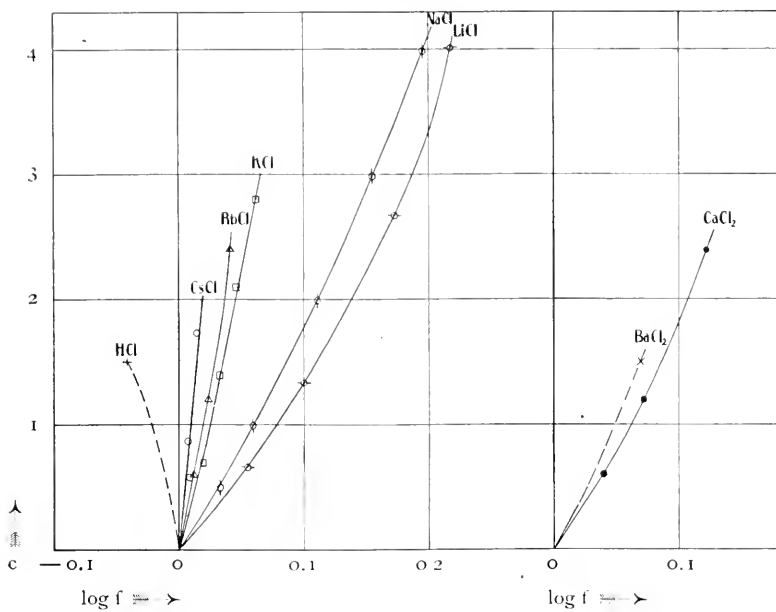
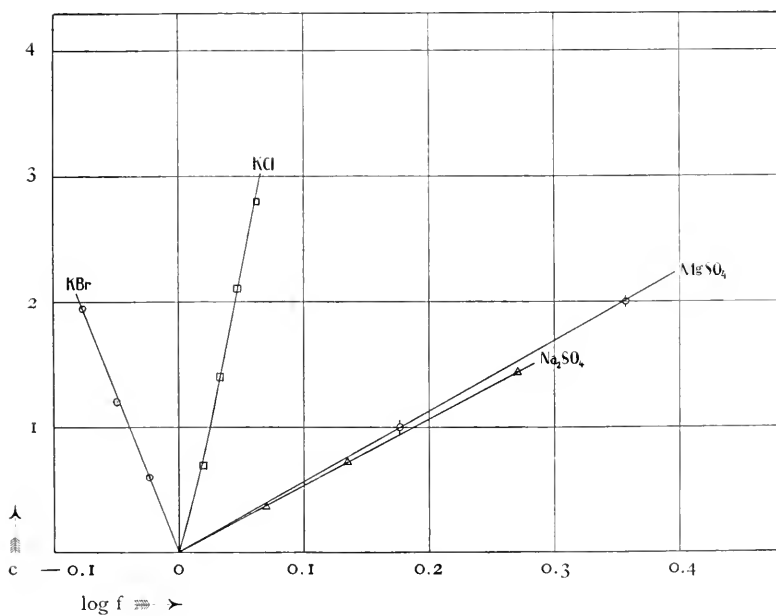


Fig. 5.



### 3. Solubility of Succinic Acid.

The succinic acid was prepared from a very pure product of Mercks, by recrystallisation; it showed the right melting point, and samples recrystallised from water and alcohol gave the same solubility. The temperatures were abt. 12°, abt. 18° and abt. 24°.

The succinic acid (which was suggested to me by Prof. Baggesgaard-Rasmussen) was titrated with 0.1 n NaOH, using phenolphthalein as indicator. For the rest, the investigation was carried out as with the two preceding substances, save that  $s_0$  here is not the solubility in 0.01 n HCl, but in pure water, and  $s_n$  the solubility in salt solution without addition of hydrochloric acid.

c                      s                      f                      log f                      k

Table 28a. LiCl. 11°.85  $\pm$  0°.02.

0	0.3854	1.000	0.0000	—
0.669	0.3062	1.259	0.1000	0.149
1.338	0.2435	1.583	0.1995	0.149
2.676	0.1558	2.474	0.3934	0.147
4.013	0.1036	3.720	0.5705	0.142

Table 28b. LiCl. 18°.

0	0.4974	1.000	0.0000	—
0.335	0.4417	1.126	0.0515	0.154
0.669	0.3950	1.259	0.1000	0.149
1.338	0.3151	1.579	0.1984	0.148
2.676	0.2018	2.465	0.3918	0.146
4.013	0.1348	3.690	0.5670	0.141

Table 28c. LiCl. 23°.75.

0	0.6344	1.000	0.0000	—
0.669	0.5037	1.260	0.1004	0.150
1.338	0.4046	1.568	0.1954	0.146
2.676	0.2590	2.449	0.3890	0.145
4.013	0.1739	3.648	0.5621	0.140

Table 29a. NaCl. 11°.85.

0	0.3854	1.000	0.0000	—
0.4	0.3584	1.075	0.0314	0.079
0.8	0.3330	1.157	0.0633	0.079
1.6	0.2853	1.351	0.1307	0.082
3.2	0.2082	1.851	0.2674	0.084

c	s	f	log f	k
Table 29b. NaCl. $18^{\circ} \pm 0^{\circ}.02$ .				
0	0.4971	1.000	0.0000	—
0.1	0.4889	1.017	0.0073	0.073
0.4	0.4611	1.078	0.0326	0.082
0.8	0.4262	1.166	0.0667	0.083
1.6	0.3602	1.380	0.1399	0.087
2.4	0.3091	1.608	0.2063	0.086
3.2	0.2625	1.894	0.2774	0.087
4.0	0.2246	2.213	0.3450	0.086

Table 29c. NaCl. $23^{\circ}.75$ .				
0	0.6344	1.000	0.0000	—
0.4	0.5853	1.084	0.0350	0.088
0.8	0.5406	1.174	0.0697	0.087
1.6	0.4597	1.380	0.1399	0.087
3.2	0.3305	1.920	0.2833	0.089

Table 30a. KCl. $11^{\circ}.85$ .				
0	0.3854	1.000	0.0000	—
0.7	0.3824	1.008	0.0035	0.005
1.4	0.3771	1.022	0.0095	0.007
2.1	0.3693	1.044	0.0187	0.009
2.8	0.3593	1.073	0.0306	0.011

Table 30b. KCl. $18^{\circ}$ .				
0	0.4976	1.000	0.0000	—
0.35	0.4913	1.013	0.0056	0.016
0.7	0.4851	1.026	0.0112	0.016
1.4	0.4713	1.056	0.0237	0.017
2.1	0.4564	1.090	0.0374	0.018
2.8	0.4413	1.128	0.0523	0.019
3.5	0.4262	1.168	0.0674	0.019

Table 30c. KCl. $23^{\circ}.75$ .				
0	0.6344	1.000	0.0000	—
0.7	0.6112	1.038	0.0162	0.023
1.4	0.5909	1.074	0.0310	0.022
2.1	0.5708	1.111	0.0457	0.022
2.8	0.5459	1.162	0.0652	0.023

c	s	f	log f	k
Table 31. RbCl. $17^{\circ}.85 \pm 0^{\circ}.02$ .				
0	0.4914	1.000	0.0000	—
0.6	0.4955	0.992	—0.0035	—0.006
1.2	0.4980	0.987	—0.0057	—0.005
2.4	0.4955	0.992	—0.0035	—0.002

Table 32. CsCl. $17^{\circ}.85$ .				
0	0.4917	1.000	0.0000	—
0.578	0.5085	0.967	—0.0145	—0.025
0.867	0.5169	0.951	—0.0218	—0.025
1.733	0.5355	0.918	—0.0372	—0.022

Table 33. KBr. $17^{\circ}.85$ .				
0	0.4928	1.000	0.0000	—
0.6	0.4938	0.998	—0.0009	—0.002
1.2	0.4899	1.006	0.0026	0.002
2.4	0.4777	1.032	0.0137	0.006

Table 34. NaJ. $17^{\circ}.85$ .				
0	0.4928	1.000	0.0000	—
0.6	0.4715	1.045	0.0191	0.032
1.2	0.4502	1.095	0.0394	0.033
2.4	0.4101	1.202	0.0799	0.033

Table 35. KJ. $17^{\circ}.85$ .				
0	0.4928	1.000	0.0000	—
0.6	0.5132	0.960	—0.0177	—0.029
1.2	0.5300	0.930	—0.0315	—0.026
2.4	0.5495	0.897	—0.0472	—0.020

From the separate measurements of  $s_0$  we get the following mean values:

$s_0$ :	0.3854	$11^{\circ}.85$
	0.4920	$17^{\circ}.85$
	0.4974	$18^{\circ}$
	0.6344	$23^{\circ}.75$ .

The accuracy is as for quinone.

As with hydroquinone, the relation between  $f_{18^{\circ}}$  and  $c$  is shown graphically in Figures 6 and 7.

Fig. 6.

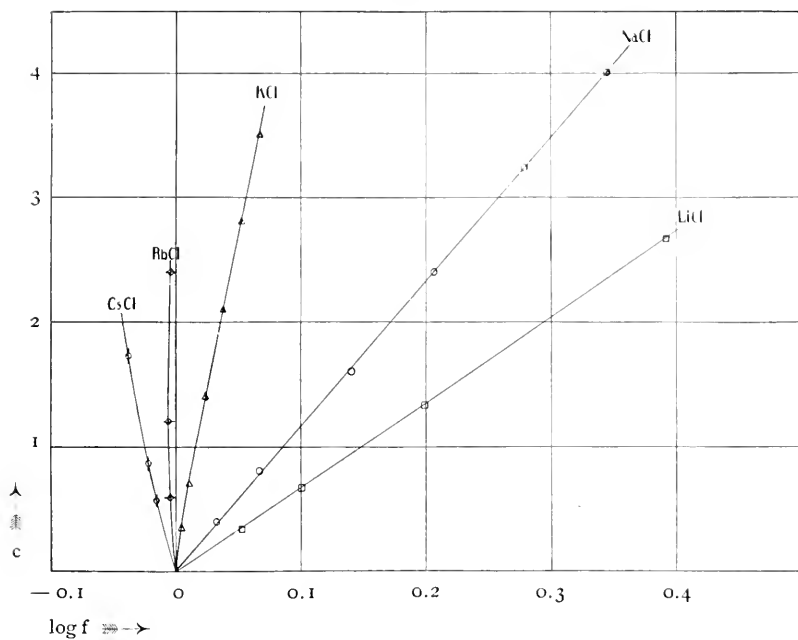
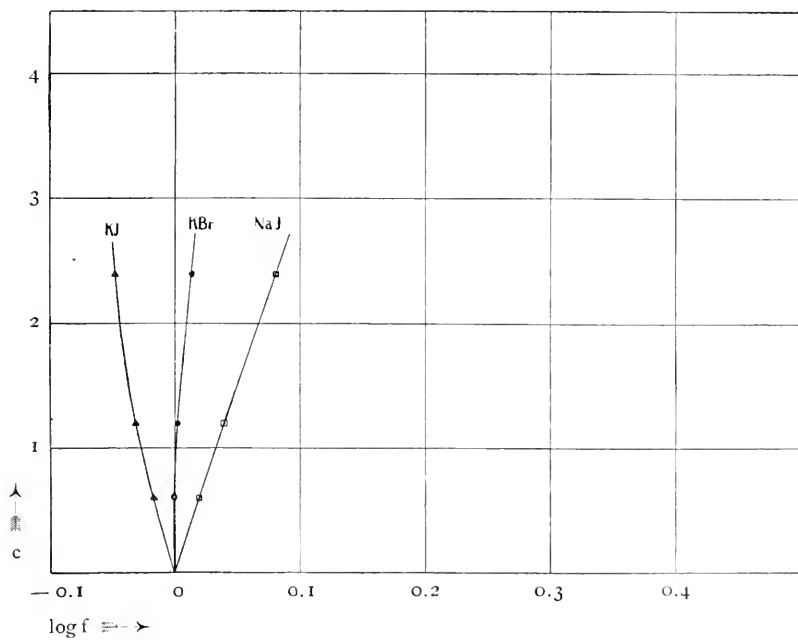


Fig. 7.



#### 4. Solubility of Boracic Acid.

Solubility determinations of boracic acid in salt solutions have been made before. Unfortunately, I have not had access to the paper in question, by P. Bogdan<sup>1)</sup>. It would seem, however, from an abstract in the Chem. Centralblatt<sup>2)</sup>, as if Bogdan had employed only the following salts:  $\text{NaNO}_3$ ,  $\text{KCl}$ ,  $\text{KNO}_3$ ,  $\text{K}_2\text{SO}_4$  and  $\text{Na}_2\text{SO}_4$ , and it is not possible to make any exact analysis of the action of salt on boracic acid by means of these. I have therefore carried out solubility determinations of boracic acid with some of the same salts mentioned before.

The preparation employed was made up by recrystallisation of a trade product. The determination was carried out as with succinic acid, save that for every 5 cc solution of boracic acid, 25 cc glycerine was added. On titration of weighed quantities of boracic acid, it was noticed that this mixture was the most suitable, when the quantity of boracic acid in the 5 cc answered to abt. 20 cc 0.2 n  $\text{NaOH}$ , and that further addition of glycerine was unnecessary. For the rest, as with succinic acid, 0.01 n  $\text{HCl}$  was likewise not added.

c	s	f	log f	k
Table 36a. $\text{LiCl}$ . $12^\circ \pm 0^\circ.02$ .				
0	0.6061	1.000	0.0000	—
0.669	0.5276	1.149	0.0603	0.090
1.338	0.4663	1.300	0.1139	0.085
2.676	0.3683	1.646	0.2164	0.081
4.113	0.2927	2.071	0.3162	0.077

Table 36b. $\text{LiCl}$ . $17^\circ.85$ .				
0	0.7219	1.000	0.0000	—
0.669	0.6379	1.132	0.0539	0.081
1.338	0.5642	1.280	0.1072	0.080
2.676	0.4492	1.607	0.2062	0.077
5.351	0.3126	2.309	0.3634	0.068

Table 36c. $\text{LiCl}$ . $23^\circ.85$ .				
0	0.8633	1.000	0.0000	—
0.669	0.7622	1.133	0.0542	0.081
1.338	0.6714	1.286	0.1092	0.082
2.676	0.5396	1.600	0.2041	0.076
4.175	0.4380	1.971	0.2947	0.071

<sup>1)</sup> Ann. scient. de l'Univers. de Jassy. 2, 95.

<sup>2)</sup> Chem. Centralblatt. 1903. 2, 1.



c	s	f	log f	k
Table 37 a. NaCl. $12^{\circ} \pm 0.02$ .				
0	0.6061	1.000	0.0000	—
0.4	0.6033	1.005	0.0022	0.006
0.8	0.6000	1.010	0.0043	0.005
1.6	0.5953	1.018	0.0078	0.005
3.2	0.5794	1.046	0.0195	0.006

Table 37 b. NaCl. $17^{\circ}.85$ .				
0	0.7219	1.000	0.0000	—
0.8	0.7116	1.015	0.0065	0.008
1.6	0.7008	1.030	0.0128	0.008
2.4	0.6889	1.048	0.0204	0.009
3.2	0.6769	1.066	0.0278	0.009

Table 37 c. NaCl. $23^{\circ}.85$ .				
0	0.8633	1.000	0.0000	—
0.4	0.8514	1.014	0.0060	0.015
0.8	0.8426	1.025	0.0107	0.013
1.6	0.8223	1.050	0.0212	0.013
3.2	0.7944	1.087	0.0362	0.011

Table 38 a. KCl. $12^{\circ}$ .				
0	0.6061	1.000	0.0000	—
0.7	0.6403	0.947	— 0.0236	— 0.034
1.4	0.6714	0.903	— 0.0443	— 0.032
2.1	0.7028	0.862	— 0.0645	— 0.031
2.8	0.7379	0.821	— 0.0857	— 0.031

Table 38 b. KCl. $17^{\circ}.85$ .				
0	0.7219	1.000	0.0000	—
0.7	0.7558	0.955	— 0.0200	— 0.029
1.4	0.7884	0.916	— 0.0381	— 0.027
2.1	0.8199	0.881	— 0.0550	— 0.026
2.8	0.8538	0.846	— 0.0726	— 0.026

Table 38 c. KCl. $23^{\circ}.85$ .				
0	0.8633	1.000	0.0000	—
0.7	0.8960	0.964	— 0.0159	— 0.023
1.4	0.9306	0.928	— 0.0324	— 0.023
2.1	0.9609	0.898	— 0.0467	— 0.022
2.8	0.9959	0.867	— 0.0620	— 0.022

c	s	f	log f	k
Table 39. RbCl. $18^{\circ} \pm 0^{\circ}.02$ .				
0	0.7319	1.000	0.0000	—
0.6	0.7689	0.952	— 0.0214	— 0.036
1.2	0.8083	0.905	— 0.0433	— 0.036
2.4	0.8880	0.824	— 0.0841	— 0.035

Table 40. CsCl. $18^{\circ}$ .				
0	0.7307	1.000	0.0000	—
0.732	0.7725	0.946	— 0.0241	— 0.033
1.455	0.8203	0.891	— 0.0501	— 0.034
2.177	0.8713	0.839	— 0.0762	— 0.035

Table 41. HCl. $17^{\circ}.85$ .				
0	0.7219	1.000	0.0000	---
0.6	0.6435	1.122	0.0500	0.083
1.2	0.5778	1.249	0.0966	0.081
1.5	0.5463	1.321	0.1209	0.081

Table 42. KJ. $17^{\circ}.85$ .				
0	0.7219	1.000	0.0000	—
0.6	0.7219	1.000	0.0000	0.000
1.2	0.7239	0.997	— 0.0013	— 0.001
1.8	0.7223	0.999	— 0.0004	0.000
2.4	0.7175	1.006	0.0026	0.001

Table 43. MgCl <sub>2</sub> . $17^{\circ}.85$ .				
0	0.7219	1.000	0.0000	—
0.495	0.6562	1.100	0.0414	0.084
0.989	0.5973	1.209	0.0824	0.083
1.979	0.4997	1.445	0.1599	0.081
3.958	0.3500	2.063	0.3145	0.079

Table 44. CaCl <sub>2</sub> . $17^{\circ}.85$ .				
0	0.7219	1.000	0.0000	—
0.598	0.6530	1.106	0.0438	0.073
1.195	0.5937	1.216	0.0849	0.071
2.390	0.4890	1.476	0.1691	0.071
4.780	0.3417	2.113	0.3249	0.068

c                      s                      f                      log f                      k

Table 45.  $\text{BaCl}_2$ .  $17^\circ.85 \pm 0^\circ.02$ .

0	0.7219	1.000	0.0000	—
0.8	0.6769	1.066	0.0278	0.035
1.2	0.6594	1.095	0.0394	0.033
1.6	0.6451	1.119	0.0488	0.031

Accuracy as for quinone. Values of  $s_0$  as follows:

0.6061	$12^\circ$
0.7219	$17^\circ.85$
0.8633	$23^\circ.85$

As with hydroquinone, relation between  $f_{18^\circ}$  and  $c$  shown graphically in Figs. 8 and 9.

Fig. 8.

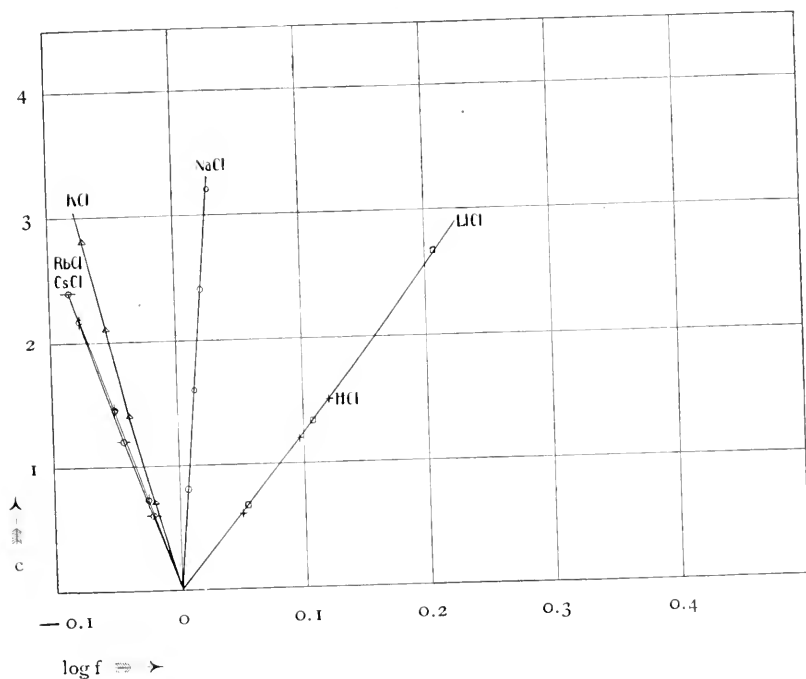
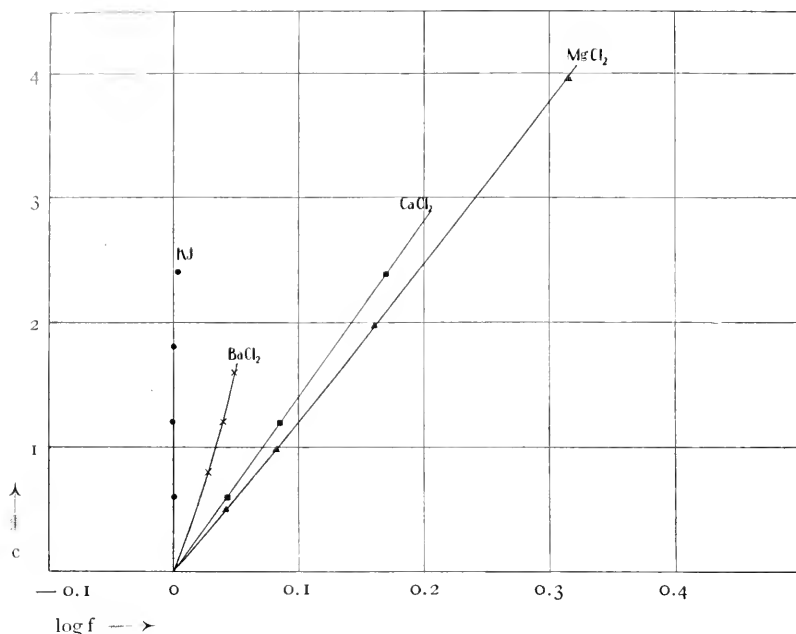


Fig. 9.



## 5. Comparison of Results.

As regards comparison with other investigations which have been made public, no determination of  $f$  has been made, so far as I am aware, for the four substances here mentioned, save those mentioned in the paper by P. Bogdan. I can therefore only compare the solubility in water with earlier measurements, and here again only for succinic and boracic acid.

With *succinic* acid, several determinations of solubility in water have been made, viz. by Carius<sup>1)</sup>, Miczynski<sup>2)</sup>, Bourgoin<sup>3)</sup>, Herz und Knoch<sup>4)</sup> and by Marshall and Cameron<sup>5)</sup>. As the temperatures at which these writers worked did not correspond with mine, I have for purposes of comparison drawn the temperature curve for solubility for the interval from 10° to 25°

<sup>1)</sup> Liebig's Ann. **142**, 146. (1867).

<sup>2)</sup> Mon. Chem. **7**, 255. (1886).

<sup>3)</sup> Bull. Soc. Chim. **21**, 110. (1874).

<sup>4)</sup> Zeitschr. f. anal. Chem. **41**, 320. (1902).

<sup>5)</sup> Journ. of Chem. Soc. **91**, 1522. (1907).

by the aid of my own determinations, and by reading from the curve determined the solubility at the temperatures in question.

Table 46.

Temperature	From the curve	Found by . . .	Observer
14.5	0.431	0.441	Bourgoin
18	0.497	0.501	Carius
20	0.544	0.540	Herz, Knoch
»	»	0.550	{ Marshall and
25	0.675	0.681	{ Cameron.

The solubility is as before noted in gram-molecules per litre. The agreement must be said to be satisfactory.

In the case of *boracic* acid, for which we have measurements by Herz und Knoch (loc. cit.) and Nasini e Ageno<sup>1)</sup> I have proceeded in the same manner.

Table 47.

Temperature	From the curve	Found by . . .	Observer
12.2	0.608	0.603	Nasini, Ageno
13	0.625	0.620	Herz, Knoch
20	0.773	0.792	» »
21	0.796	0.801	Nasini, Ageno
25	0.896	0.900	Herz, Knoch.

Some measurements by Ditte<sup>2)</sup> which lie quite outside those here given, I have not included here.

## 6. Heat of Solution.

Before going further into the theoretical side of the matter, I will calculate the heat of solution from the solubility at the different temperatures. The alteration of this last with the salt concentration, which must stand in a certain relation to the corresponding alteration in  $f$ , can be approximately determined from the curves for  $f$  at  $12^\circ$ ,  $18^\circ$  and  $24^\circ$ , being connected with the variation of  $f$  with the temperature in the following manner:

<sup>1)</sup> Zeitschr. f. phys. Chem. **69**, 482. (1910).

<sup>2)</sup> Ann. Chim. phys. (5) **13**, 67. (1878).

If the solubility of the neutral substance S be slight, then the heat of solution  $U_o$  in pure water (or 0.01 n HCl) is determined by:

$$-U_o = RT^2 \frac{d \ln s_o}{dT}, \quad (9)$$

and in the salt solution of concentration  $c_n$

$$-U_n = RT^2 \frac{d \ln s_n}{dT}. \quad (10)$$

On considering a differential transference of one gram-molecule S from water to salt solution, and using the expression:

$$A - U = T \frac{dA}{dT},$$

we obtain

$$U_n - U_o = RT^2 \left( \frac{d \ln f}{dT} \right)_c, \quad (11)$$

an expression which can also be arrived at by subtraction of (10) from (9) (cf. also J. N. Brønsted: »Über die Temperaturabhängigkeit der Löslichkeit und der Aktivitäts- und osmotischen Koeffizienten von Salzen«<sup>1)</sup>).

I will, however, as far as possible, avoid going into the question of relation between alteration of the heat of solution and that of the work of solution with the concentration, as this question is still very little elucidated, and the measurements here made of  $U$  call for comparison with direct calorimetrical, more accurate determinations.

Only with hydroquinone and quinone, which are here subjected to special treatment, will I calculate an approximate value of  $U_n - U_o$  at the salt concentration 1 n, although in the case of hydroquinone certain circumstances have to be considered which render these values somewhat uncertain, and which will be dealt with in a later work.

From (9) we obtain, by integration, and assuming  $U_o$  to be independent upon the temperature:

$$U_o \left( \frac{1}{T''} - \frac{1}{T'} \right) = R \cdot \ln \frac{s'_o}{s''_o} \quad (12)$$

from which  $U_o$  can be found.

a) Hydroquinone:

$$U_o = -5710 \text{ cal.}$$

<sup>1)</sup> Zeitschr. f. phys. Chem. **100**, 139. (1922).

A direct determination by Forcrand<sup>1)</sup> gives  $U_o = -4420$  at  $20^\circ$ . The agreement is bad. The cause of this should presumably be sought in the remarkable features attending the solubility of hydroquinone in the vicinity of these temperatures, as at  $12^\circ$  I did not succeed in getting a well defined saturation. These conditions suggest the possibility of a transition point, perhaps two, in the neighbourhood of  $18^\circ$ . I will not, however, go further into this, but merely point out the uncertainty in determination of  $U_o$  and  $U_n$  which it involves.

Table 48 shows  $U_n$  calculated for  $c = 1$  from (11).

Table 48.

Salt	$\log f_{24} - \log f_{18}$	$T \frac{\Delta \log f}{\Delta T}$	$U_n - U_o$	$U_n (c = 1)$
LiCl	-0.0019	-0.092	-122	-5832
NaCl	0	0	0	-5710
KCl	0	0	0	-5710
CsCl	+0.0022	+0.107	+142	-5568
CaCl <sub>2</sub>	-0.0041	-0.199	-265	-5975
BaCl <sub>2</sub>	0	0	0	-5710

## b) Quinone:

$$U_o = -4810. \quad 11^\circ.85-18^\circ.$$

$$U_o = -5300. \quad 18^\circ-23^\circ.80.$$

$$U_o = -5050. \quad 11^\circ.85-23^\circ.80.$$

$$\text{Surest Value of } U_o = -5050 \text{ cal.}$$

Table 49.

Salt	$\log f_{24} - \log f_{18}$	$T \frac{\Delta \log f}{\Delta T}$	$U_n - U_o$	$U_n (c = 1)$
LiCl	+0.0052	+0.252	+335	-4715
NaCl	0.0063	0.306	407	-4643
KCl	0.0072	0.349	464	-4586
KBr	0.0144	0.698	928	-4122
Na <sub>2</sub> SO <sub>4</sub>	0.0070	0.340	452	-4598
CaCl <sub>2</sub>	0.0100	0.485	645	-4405

## c) Succinic acid:

$$U_o = 6830. \quad 11^\circ.85-18^\circ.$$

$$U_o = 7250. \quad 18^\circ-23^\circ.75.$$

$$U_o = 7030. \quad 11^\circ.85-23^\circ.75.$$

$$U_o = -7030 \text{ cal.}$$

<sup>1)</sup> Ann. de Chim. et de Phys. (6) **30**, 69. (1893).

An indirect measurement of  $U_o$  based on Marshall and Cameron's solubility determinations (loc. cit.) gives the value:  $-7410$  cal. A direct value by Jul. Thomsen<sup>1)</sup> is  $-6680$  cal.

d) Boracic acid:

$$U_o = -4920. \quad 12^\circ - 17^\circ.85.$$

$$U_o = -5110. \quad 17^\circ.85 - 23^\circ.85.$$

$$U_o = -5010. \quad 12^\circ - 23^\circ.85.$$

$$U_o = -5010 \text{ cal.}$$

Herz and Knoch's measurements give the two following values from  $13^\circ$  to  $20^\circ$  and from  $20^\circ$  to  $25^\circ$ :  $-4450$  and  $-5800$ . Nasini and Ageno's give:  $-5370$  cal.

## B. THEORETICAL SECTION.

### 1. General Theory.

#### *Introductory Remarks.*

The activity coefficient  $f$  is, as already mentioned, an expression of the fact that other forces act on the substance  $S$  in a salt solution than in pure water. In transporting a single molecule of  $S$  from a point dominated solely by the fields of force from the water molecules, to a point where a certain number of water molecules are replaced by ions, we can gain or carry out a work. In order to determine  $f$  by molecular-kinetic means, it is necessary to determine how this work depends upon the molecules of the system and their mutual forces, and on the temperature. When this is done, the equation of state for the substance  $S$  in the combination  $S$ , salt and water is found, and  $f$  can be calculated for any salt concentration and any temperature.

The difficulties attending such an analysis however, are extraordinarily great. They are connected, partly with circumstances which have up to the present rendered it impossible to formulate any equation of state for liquids, partly with the question as to nature of the forces here at work, the influence of chemical affinity on the constitution of the solution.

<sup>1)</sup> Berichte d. deutsch. chem. Gesellsch. **6**, 713. (1873).

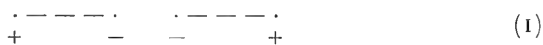


That part of the activity question for neutral substances which is connected with the equation of state for liquids I will touch on but lightly here. On the other hand, I shall endeavour to set forth some new views regarding the nature of molecular forces, applied within the range of molecular density approximately commanded by van d. Waals' equation of state, and by these views, elucidate one of the sides of the nature of the solutions and of the mutual influence of different substances in aqueous solution.

### *Nature of Molecular Forces.*

The views on the subject of the nature of molecular forces on which I base the following observations are taken from a work by P. Debye<sup>1</sup>). He himself uses the following simple illustration.

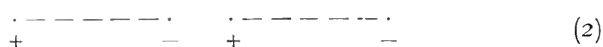
Given a non-ideal gas with molecules of the structure  $\begin{smallmatrix} \cdot & - & - & - & \cdot \\ & + & & & - \end{smallmatrix}$  an electrical dipole. If this gas follows van d. Waals' equation, the molecular forces will not alter the distribution of the molecules, their position relative to one another, and this will be seen in the fact that the potential energy is independent of temperature. If we were to suppose that the atomic systems of the molecules were perfectly stiff, that the electric parts could not be displaced in their relative positions under the influence of an extraneous electric field, then, under these conditions, the mean value of the attracting force, taken for a long period of time, would be 0. Considering two positions of our molecules:



we see that the sum of the force in the first and the second position is nil. But from the foregoing, position (1) should be just as probable as position (2), which of course is only exactly correct in the case of extremely small mutual forces or extremely high temperatures. Approximately (and the more correctly as temperature increases) we can reckon all positions as equally justifiable. We can therefore divide them into such corresponding pairs of positions, where the sum of the forces is 0. The mean value of the forces for all positions must therefore be 0.

<sup>1</sup>) Phys. Zeitschr. **21**, 178. (1920).

It is another matter however, if the molecules can not be regarded as stiff, but should be imagined as capable of polarising one another:



It will be seen from the figure that in case (1) the repulsive force is less than above, while in position (2) the attractive force is greater than above. Therefore, when we take the mean value of all positions, the main effect will be as before, but there arises an additional effect which, it will be seen, takes the form of an attractive force. This force then, must be van d. Waals' cohesive force, the limit force for high temperatures.

Besides cohesive forces, there are also repulsive forces at work between the molecules, acting only when the molecules are extraordinarily near to one another and growing without limit, when the molecules touch one another, circumstances answering to those which arise when two hard elastic balls collide with one another. These forces also find their explanation in the structure of the molecules, as set forth by Born in his work on crystal structure.

With the interplay between forces of this sort, then we must imagine the potential energy arising in a non-ideal gas, following van d. Waals' equation, or the following expression:

$$\left(p + \frac{a}{v^2}\right) v = RT \left(1 + \frac{b}{v}\right), \quad (13)$$

which at the first approximation agrees with van d. Waals'<sup>1)</sup>.

$\frac{a}{v^2}$  is here the »internal pressure« which, according to Debye's view, can be determined from the structure of the molecules,  $b$  a measure of the repulsive forces, viz. four times the volume of the molecules themselves in one gram-molecule. This equation I shall in the following take as the basis for determination of  $f$  for gases and mixtures of gases, disregarding the chemical influences between the molecules, association. (For dipoles, see Debye loc. cit.).

<sup>1)</sup> See for instance Jeans: The Dynamical Theory of Gases. pag. 133.

*f for Gases.*

In the case of a non-ideal gas, an expression for  $f$  can easily be derived by transferring one gram-molecule from a large reservoir with pressure  $p$  to another with pressure  $p + dp$ . The work is  $vdp$ , definitionally equal to  $RT \, d \ln s \cdot f$  whence by simple calculation we get:

$$\ln f = 2 \left( b - \frac{a}{RT} \right) \cdot s \quad (14)$$

$$\text{or } \log f = 0.8686 \left( b - \frac{a}{RT} \right) \cdot s, \quad (15)$$

where  $s$  is the number of gram-molecules per litre.

We see then that by using (13) we obtain an expression for  $f$  agreeing in form with (1), and in such a manner that  $b$ , which is an expression of the repulsive forces, gives an activity coefficient increasing with the concentration, whereas  $a$  acts in the opposite direction, a result which was of course to be expected.

If the system contains more than one component, then, as mentioned in the introduction, it is simplest to let the one considered be of slight concentration. If the remaining components  $S_1 S_2 \dots S_n$  be found at the concentrations  $c_1 c_2 \dots c_n$  we can then write (13) as follows:

$$\left( p + \frac{2 \sum a_i \cdot c_i}{v} \right) \cdot v = RT \cdot (1 + 2 \sum b_i \cdot c_i)^1 \quad (16)$$

where  $p$  is the pressure,  $v$  the molal volume of  $S$  and  $a_i$  and  $b_i$  constants characteristic of the interaction between  $S_i$  and  $S$ .

From this equation we get:

$$\frac{d \ln f}{dc_i} = 2 \left( b_i - \frac{a_i}{RT} \right),$$

an expression which determines the variation of  $f$  with one of the components when the remainder are kept constant. If the alteration in  $S_i$  be combined with corresponding alteration in another component,  $S_r$  for instance, so that the molecules of  $S_r$  are successively replaced by molecules of  $S_i$ , then we obtain:

$$\frac{d \ln f}{dc_i} = 2 \left( (b_i - b_r) - \frac{a_i - a_r}{RT} \right). \quad (17)$$

<sup>1)</sup> This generalisation is based on the additivity of the cohesive effect, and is thus in agreement with the foundation of both van d. Waals' and Debye's mode of observation. The closer derivation is a simple parallel to that given by van d. Waals.

The total energy which can be gained by transference of  $S$  from  $p$  to  $p + dp$ , can be determined by (11):

$$dU = 2a \cdot ds,$$

$b$  and  $a$  being independent of temperature.

*Calculation of the constants  $a$  and  $b$ .*

I will not here go further into the derivation of  $a$  in Debye's work, but merely point out the following:

If the electrical force (arising from the molecules) at that point of the gas where a molecule under observation happens to be, is  $E$ , Debye then sets the polarisation of the molecule as equal to

$$-\frac{a}{2} \cdot E \text{ and the potential therefore equal to } -\frac{a}{2} \cdot E^2.$$

$a$  can be found from the molecular refraction of the gas at the frequency 0, which is a measure of the polarisation of the molecule in the electric field of the light wave. And when we calculate the mean value of the square of the electric force<sup>1)</sup> in a point inside the gas, then the potential energy of a single molecule is evidently equal to  $-\frac{a}{2} \cdot \overline{E^2}$ , from which, by summing up the potentials of all the molecules, we obtain  $U$ , the potential energy of the gas, from which again we can calculate the constant  $a$  by the equation:

$$-U = a \cdot s \text{ where } s \text{ is the concentration of the gas.}$$

Debye now determines  $\overline{E^2}$  under the presupposition that the molecules are not, as mentioned in the illustrating example, dipoles, as these are inclined to associate; he assumes that they are molecules in which the centre of gravity for the positive electric charges coincides with that for the negative, i. e. quadrupoles, to which precisely the same observation applies as on p. 29. The result of the calculations gives the following value for  $a$ :

$$a = \frac{9}{10} \cdot P_0 \cdot N \cdot \frac{t^2}{d^5}, \quad (18)$$

where  $P_0$  is the molecular refraction,  $N = 6.06 \cdot 10^{23}$ ,  $t$  the mean momentum of inertia of the electric charges in the molecule and  $d$  the diameter. Forces of this character between quadrupoles are inversely proportional to a very high power of the distance,

<sup>1)</sup> Mean value of  $E$  is 0.

the 9th power, and the molecules determining the potential of a single observed molecule are therefore only those lying nearest to the same, a feature which must be supposed to be of great importance in consideration of the solutions.

The constant  $b$  can be found in the usual manner from the expression:

$$b = \frac{2}{3} \cdot N \cdot \pi \cdot d^3 \quad (19)$$

These equations apply to pure gases. For the forces between two kinds of molecules however, we can from Debye's theory arrive at exactly similar expressions by a simple consideration, — supposing that the centres of the molecules in question can approach so near each other as the sum of their radii, calculated from the critical volume of the pure gases, indicates.

We find, for the molecule species 1 and 2:

$$a_{1-2} = \frac{14.4}{(d_1 + d_2)^5} \cdot N \cdot (P_{o1} \cdot t_2^2 + P_{o2} \cdot t_1^2) \quad (20)$$

$$b_{1-2} = \frac{1}{12} \cdot N \cdot \pi \cdot (d_1 + d_2)^3 \quad (21)$$

I will now use these two equations to calculate  $f$  for mixtures of certain gases, making use of some of Debye's figures.

*f for Hydrogen and Pentane mixed with Helium, Argon, Krypton and Xenon.*

I reproduce here the values required for calculation:

Table 50.

Gas	$T_k$	$p_k$	$a \cdot 10^{-12}$	$b$	$d \cdot 10^8$	$P_o$	$t \cdot 10^{2.6}$
H <sub>2</sub>	32	19.4	0.15	16.6	2.36	2.03	3.20
C <sub>5</sub> H <sub>12</sub>	470	33.0	19.2	145	4.85	25.7	60.7
He	5	2.8	0.026	18.6	2.44	0.52	2.84
Ar	151	48.0	1.37	32.2	2.93	4.17	11.2
Kr	210	54.3	2.34	39.6	3.14	6.25	14.5
X	288	57.2	4.17	51.6	3.42	10.2	18.9

$T_k$  and  $p_k$  are critical temperatures and pressures, the last in atmospheres, the remaining quantities in absolute measure,  $a$  in dyn cm<sup>4</sup>,  $b$  in cm<sup>3</sup>,  $d$  in cm.  $P_o$  has the dimensions cm<sup>-3</sup>,  $t$  the dimensions g<sup>1/2</sup> · cm<sup>7/2</sup> · sec<sup>-1</sup>. It will be noticed, how greatly  $t$  and  $p_o$  increase with the size and complication of the molecule.

In mixtures of for instance a very small quantity of hydrogen in He, Ar, Kr. and X, the activity coefficient for the hydrogen is determined by  $\log f = 0.8686 (b_i - \frac{a_i}{RT}) \cdot c_i$ ,

$c$  being the concentration of one of the four gases mentioned,  $a_i$  and  $b_i$  the constants for interaction between it and the hydrogen — determined by (20) and (21). The quantity

$$k = 0.8686 (b_i - \frac{a_i}{RT})$$

is thus a constant characteristic of the "solution" of hydrogen in this gas, a measure of the activity effect. This constant I have therefore calculated for hydrogen and pentane in mixtures with helium, argon, krypton and xenon. The values will be found in Table 51.

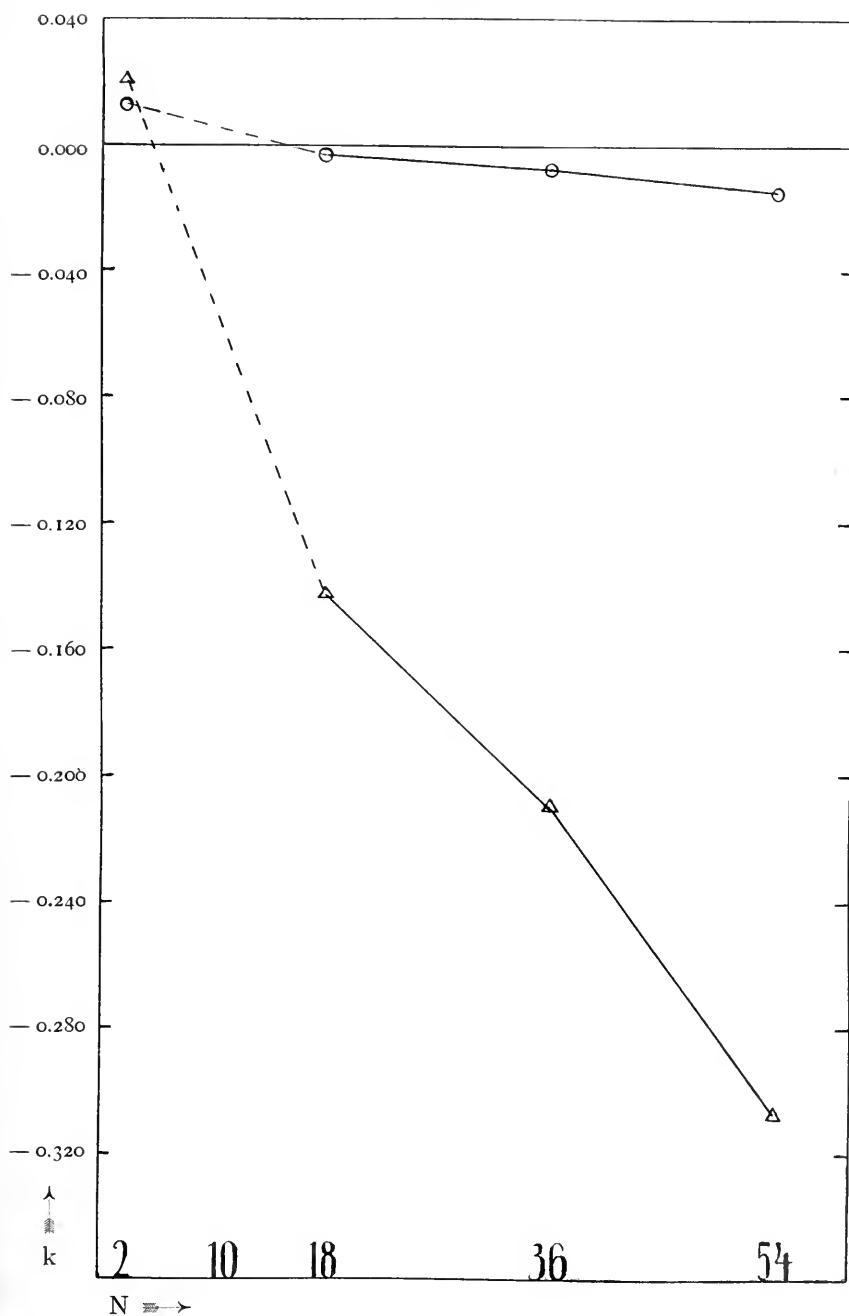
Table 51.	Hydrogen			Pentane		
	$a_i \cdot 10^{-12}$	$b_i$	$k$	$a_i \cdot 10^{-12}$	$b_i$	$k$
Helium	0.075	0.018	0.013	0.899	0.062	0.021
Argon	0.628	0.024	-0.003	5.69	0.075	-0.143
Krypton	0.851	0.026	-0.008	7.64	0.081	-0.210
Xenon	1.122	0.031	-0.015	10.54	0.090	-0.308
	$\text{dyn cm}^4 \cdot 10^{-12}$	Litre	Litre	$\text{dyn cm}^4 \cdot 10^{-12}$	Litre	Litre

In order to give a clear view of these results I have in Fig. 10 plotted  $k$  as ordinate against the atomic numbers of the inactive gases as abscissa. The round points are the values for hydrogen, the triangular ones those for pentane.

The two curves are, to my mind, instructive. They show how greatly the attractive effect exceeds that of repulsion and rises (answering to diminishing values of  $k$ ) with the magnitude of the molecules considered, both those affected and those producing the effect. We can therefore conclude from the theory that if we had determined, experimentally,  $f$  for the mixture of a gas with helium, argon etc. and found a markedly falling curve from helium to xenon, then the cohesive action between the molecules of this gas and those of the inactive gases must be great; a conclusion which will prove of importance later on.

In this connection, there is a question we must touch on, and which throws new light on these curves. We know that according to the periodical system there must be a certain rela-

Fig. 10.



tionship between for instance the chlorine ion, argon and the potassium ion, as potassium, by giving off its electron, obtains an electron system having the same configuration as that of argon, and chlorine, by catching an electron, likewise. The difference lies solely in the magnitude of the orbits, which depend on the nuclear charge and resulting difference in volume and polarisability. In the same way there is a relationship between the sodium ion, neon and the fluorine ion — the rubidium ion, krypton and the bromine ion — the caesium ion, xenon and the iodine ion. We can thus from these curves (Fig. 10) draw some conclusions as to the cohesive forces of the ions.

### *Solutions of Ions.*

If we take, from a work by Heidweiller<sup>1)</sup>, the characteristic quantities  $P_0$  and  $d$  for the ions, together with the corresponding for the inactive gases, the result will be as follows:

Table 52.

	Ion <sup>-</sup>		Inactive Gas		Ion <sup>+</sup>	
	$d \cdot 10^8$	$P_0$	$d \cdot 10^8$	$P_0$	$d \cdot 10^8$	$P_0$
N =	—	—	2		3	
			2.44	0.52	1.75	1.75
N =	9		10		11	
	—	—	—	—	1.93	2.33
N =	17		18		19	
	2.74	6.69	2.93	4.17	2.38	4.36
N =	35		36		37	
	3.15	10.22	3.14	6.25	2.62	5.73
N =	53		54		55	
	3.70	16.35	3.42	10.20	2.93	8.22

The distribution of the salt's molecular refraction between the two ions is, however, according to Heidweiller, subject to a certain arbitrary variation, as in calculating the refraction of the separate ions, he makes use of the supposition that the ratio between the refractions of the hydrogen ion and the chlorine ion is equal to that of the refractions of hydrogen and chlorine, which is very doubtful. I therefore give some figures from Wasa-

<sup>1</sup> Ann. d. Phys. **41** (4), 499 (1913).



stjerna<sup>1)</sup>, calculated on the supposition that the refraction of the hydrogen ion is 0, this supposition being in accordance with modern views as to the structure of the ions.

Table 52 a.

Ion =	Na <sup>+</sup>	K <sup>+</sup>	Rb <sup>+</sup>	Cs <sup>+</sup>	Cl <sup>-</sup>
P =	0.74	2.85	4.41	7.36	8.45.

It will be seen that Wasastjerna's view, in relation to Heidweiller's, ascribes to the negative ion a larger portion of the salt's refraction, whereby the fall in P from negative ion via inactive gas to positive ion:

	Cl <sup>-</sup>	Ar	K <sup>+</sup>
P =	8.45	4.17	2.85

becomes greater.

The question as to size of the ions has not yet been cleared up, and I will therefore let Heidweiller's figures stand as an illustration, at the same time referring to another work by Lorenz<sup>2)</sup> in which the point is thoroughly dealt with.

There is, then, as was to be expected, a numerical basis for the application of these observations to aqueous solutions of ions. That this is nevertheless attended with extraordinary difficulties, is due to the following cause or causes.

Let us consider a molecule of a neutral substance S, dissolved at a slight concentration in water. Owing to the effect of the electric fields from the surrounding water dipoles<sup>3)</sup>, it possesses a certain potential energy depending on the density of these, and also upon certain constants characteristic for both sorts of molecules. We will now successively replace the water molecules by salt ions, two by two, e. g. by KCl, the ions of which, apart from their plus or minus sign, we will take as equal. The potential energy of our molecule will then be altered, and for four reasons.

Firstly, owing to the decreasing of the field from the water molecules with the field from those we have removed, secondly owing to the electric field from the charges of the ions we have set in their place, thirdly owing to the cohesive forces from these ions, and fourthly and lastly, because the total molecular density

<sup>1)</sup> Översikt av Finska Vetensk. Soc. Förhandl. **63**, Avd. A. Nr. 4 (1921).

<sup>2)</sup> Raumerfüllung und Ionenbeweglichkeit. (Voss) Leipzig. 1922.

<sup>3)</sup> The characteristic quantity for these fields is not  $t$ , the momentum of inertia, but the moment of the electric charges.

has been altered by the interaction taking place between water and salt (number of molecules plus ions per unit of volume is not the same as the initial number of water molecules). All this supposing that no chemical reactions take place, that the water is not associated, the salt not hydrated. Disregarding for the present the alteration in molecular density, we are evidently getting round the difficulty attending investigation of the constitution of fluids, and keeping to that of solutions, as we can regard the number of water molecules per unit of volume as constant, and instead regard the question as if we had added a hypothetical substance the effect of which would be the difference between the action of the ions and that of the water molecules, a view which lies implicitly in the equation (17).

. Now, however, another question arises. What is the ratio between the influence of the ionic charge and the effect of the structure of the ions in the value of our molecule's potential? This question is of great importance, but unfortunately, it is connected with the importance of the dielectricity constant of the water to these forces, which is very difficult to determine in concentrated solutions, where it would hardly be permissible to regard the water as a continuous dielectric. It would seem however, as if the dielectric affected the forces from the charges more than it does the forces of cohesion.

When a molecule S is placed, as mentioned, in a volume of water, it will be under a certain influence from the surrounding water molecules. Owing to the dielectric qualities of these, we must now expect that only those immediately adjacent would be of importance in this respect, as they would weaken the electric field from the others. And owing to the molecular structure of the water, — which cannot be regarded as a continuously extended dielectric — it is possible that the field of these very nearly adjacent molecules must act with its full value, and that there will only be a weakening of the field at distances which permit other dipoles to intervene. In such a discontinuous dielectric therefore, it is likely that an interaction between altogether arbitrary particles following a certain law of force will be of greater effect the higher a negative power of their distance this law of force contains, i. e. the smaller the error involved in disregarding particles situated farther off. When therefore we carry our molecule S from the water to a salt solution, we will only

have to consider the very nearest of the water molecules and the replacing ions, as the forces from the ionic charges and still more the cohesive force of the particles farther off will be impaired. But at the same time, the error incurred in disregarding the effect of the dielectric is much smaller in the case of the cohesive forces than in that of the forces of ionic charges<sup>1)</sup>. Whether this mode of view can stand severe criticism I will leave to be seen; the difficulty lies, as we shall see, in the question as to what value of the dielectricity constant for the water we are to reckon with, a question which is very complicated when dealing with concentrated solutions and forces acting at such short distances. (For saturation of the dielectric see Debye: "Zur Theorie der Elektrolyte"<sup>2)</sup>).

But on the basis of our point of view, it is evident that in concentrated solutions, the cohesive action will to a very high degree supersede the effect of the ionic charges, especially at such high concentrations, as we have to deal with in determinations of the salting out effect. (The distance between the ions is here of the same order of magnitude as the ions themselves). Altogether, it seems to me evident that the structure of the particles, their specific electric constitution, must at such slight distances be predominant in the development of the potential surrounding them. As we shall see later on, the experimental results support this view, as the variation in the salting out effect with the nature of the salt runs parallel with that variation in the cohesive effect of the ions which was to be expected.

It is another question, however, whether we can, at such high concentrations, even disregarding the formation of chemical complexes, consider the problem on the basis of an equation so merely approximate as van d. Waals'. As, however, I am not here aiming at any quantitative investigation, and as the validity of equation (1) at any rate justifies the isothermic treatment of the salting out effect on this basis, I will not enter into the question as to influence of molecular forces and ionic charges on the distribution in space and the relative position of the molecules. I will merely point out that the constants  $a$  and  $b$  become dependent on temperature when this factor is considered, whereby the connection between  $U$  and  $f$  becomes of a compli-

<sup>1)</sup> As these are inversely proportional to the distance in a much lower power.

<sup>2)</sup> Phys. Zeitschr. **24**, 185 (1923).

cated character — and that Reinganum's equation of state perhaps affords the basis for a more exact analysis.

As matters stand, then, we must start from equation (1),

$$\log f = k \cdot c_n$$

an equation which was probably first formulated empirically by Setschenov, and which, by the way, also appears from some theoretical observations in a work by Nernst<sup>1)</sup>. So far as we are able to disregard the alteration in molecular density and the formation of chemical compounds generally<sup>2)</sup>  $k$  may be made clear by the equation (17), as

$$k = 2 \left\{ ((b^+ + b^-) - 2b_v) - \frac{a^+ + a^- - 2a_v}{RT} \right\},$$

where  $+$  and  $-$  refer respectively to the positive and negative ions, and the index  $v$  is attached to the water molecules. If therefore we determine  $k$  for a neutral substance in solutions of the following salts: LiCl, NaCl, KCl, RbCl and CsCl, we should then be able to find the absolute variation of

$$k_2 = 2 \left( b^+ - \frac{a^+}{RT} \right)$$

with the character of the positive ion, as the negative ion is uniform throughout the series, and on the other hand, in investigations with salts such as KCl, KBr, KJ, to determine the variation of

$$k_3 = 2 \left( b^- - \frac{a^-}{RT} \right)$$

with the character of the negative ion, the positive here being uniform throughout the series. If we then could ascribe to the ions the magnitude which is determined, for instance by optical means in Table 52, then we should expect the effect of cohesive force on the variation of  $k$  to run parallel from KCl to KJ, argon to xenon and from KCl to CsCl<sup>3)</sup>. The only factor which can disturb this parallelism is the hydration of the ions. I will therefore make some brief mention of this.

<sup>1)</sup> Zeitschr. f. phys. Chem. **38**, 487 (1901).

<sup>2)</sup> Here including formation of neutral salt molecules, the strong electrolytes being regarded as completely dissociated.

<sup>3)</sup> In connection with the following it is not without interest to note that the influence of the ionic charge also must show a similar parallelism, save that the effect will fall from KCl to CsCl, etc. answering to rising values of  $k$ .

*Hydration of Ions.*

Water has as we know an affinity to the alkali ions, rising towards the lithium ion; we must imagine that not only the hydrogen ion can be captured by it (as  $\text{NH}_3$  captures a hydrogen ion?), but also other ions which can come so near, or whose electric field is so strong that a kind of stabilisation can take place. Where a true hydration begins we do not know with certainty, as neither of the methods which can determine it, the transference method and the activity method, is entirely to be relied on. Calculations by Bjerrum<sup>1)</sup>, according to the activity method with some measurements by Ellis for instance, have led to the result that the hydrogen ion is hydrated with 8 molecules of water, whereas determinations by F. Schreiner<sup>2)</sup> give 10—11 molecules. These figures seem to me abnormally high, and in point of fact, the method of calculation is also subject to doubt. It is based on the supposition that the activity of the hydrated hydrogen ion is determined solely by the electrostatic attraction between the charges of the ions, even at concentrations up to 4 normal — a supposition which it is very difficult to maintain when we consider the importance of the repulsive and cohesive forces. That we find by this method a hydration constant with varying salt concentration only shows that the equation  $\log f = k \cdot c$  for the salting out effect also applies to the ions, as

$$\log \frac{p_0}{p} = k \cdot c$$

is valid for many salts.  $p_0$  and  $p$  are the vapour pressures for water and salt solution respectively (as to the calculation see<sup>1)</sup>).

With the transference method, the electroendosmosis is a dangerous source of experimental error if membranes are used, and the addition of an alien neutral substance must, it seems to me always be the same as introducing new and unknown effects. When, owing to these conditions, the transference method gives varying hydration figures, and we compare this with what has been said above anent the activity method, it seems to me most reasonable to trust those modern methods which seek to explain the slight ionic conductivity of the small alkali ions by the turning

<sup>1)</sup> "On the activity-coefficient for ions." Medd. f. K. Vetens. Nobel. 5, Nr. 16. (1919).

<sup>2)</sup> According to private information from Dr. Schreiner regarding some investigations not yet published.

of the dipoles of the water in their strong electric field (Born, Lorenz) without having recourse to the chemical auxiliary hypothesis, that of hydration.

I will therefore later, in calculating the molecular density, disregard the hydration, or, at the outside, reckon with a hydration of the Li-ion with one molecule, which is to some extent justified by the shape thus given to the curve (Fig. 11) for molecular density.

The only thing we can say with certainty about the hydration of alkali ions and halogene ions is, that it is of no importance to the larger ions  $K^+ - Cs^+$  and  $Cl^- - J^-$ , and that we can in their case reckon with diameters answering to those of the corresponding inactive gases. As regards the divalent ions on the other hand, we must from their salt hydrates conclude that they are hydrated, also in solution, and we must expect that in the first place, the size would thus naturally increase, secondly, however, also, that the cohesive force would decrease and be to an essential degree effaced, as it can be seen from equation (18) which contains the molecular diameter in the fifth power in the denominator, that a, by doubling the diameter, already becomes 32 times less. This is of great importance in explaining the salting out of hydroquinone. The same naturally also applies when the neutral substance is hydrated, but here we have also to reckon with another point, to wit, the influence exerted thermodynamically by the variation of the vapour pressure of the water on the addition of salt upon the equilibrium between the hydrate and the solid neutral substance, a question which I shall touch upon later.

But if then, neither the ions nor S be hydrated, which from the foregoing we cannot suppose to be the case for the K-, Rb-, Cs-, Cl-, Br- or J-ion, we must expect the ions to appear in their true size, whence it follows that the parallelism pointed out in the foregoing section really must exist, though only of course when disregarding the influence of chemical affinity. With regard to this we must further say that it will to a very high degree depend upon whether the ionic charge be positive or negative and on the ionisation tendency of the ion. As the influence of these would have to be investigated in the case of every separate neutral substance, there is reason to call a curve for the dependence of  $k$  upon the nature of the ions, normal when it runs

parallel from KCl to CsCl and from KCl to KJ<sup>1)</sup>, as such a course is characterised by certain general qualities in the ions and molecules under observation, while a curve for  $k$  would be called anormal, when its course is essentially different from KCl to CsCl and from KCl to KJ, as the plus or minus sign of the ionic charge as a specific factor here disturbs the parallelism.

Before proceeding to compare the experimental material with these observations, and dealing with the normal and the anormal curve, I will just touch on the question of alteration in the molecular density.

### *Molecular density.*

In a salt solution of concentration  $c$ , the number of ions plus the number of water molecules per litre will be:

$$a = 2c + \frac{1000 \cdot d_s - c \cdot M}{18.02},$$

disregarding hydration and association.  $d_s$  is the specific gravity of the solution, and  $M$  the molecular weight of the salt.

a I have taken from Baxter & Wallace's specific gravity determinations<sup>2)</sup> calculated for LiCl — CsCl, KBr and KJ, and set forth in Tables 53—60 together with the contraction per gram-molecule of salt during solution. The specific gravities are throughout 25°/4°.

$c$	$d_s$	$d_w$	Contract. $\text{cm}^3$	$c \cdot M$	$a$	$a$ Water
Table 53. LiCl.						
0.6826	1.01361	0.99707	— 2.28	28.94	56.031	55.343
1.3716	1.02961	—	— 1.78	58.16	56.665	—
2.7577	1.06042	—	— 1.02	116.93	57.885	—
6.8822	1.14744	—	+ 0.11	291.81	61.257	—

Table 54. NaCl.						
0.5110	1.01771	—	— 8.94	29.87	55.853	—
1.0273	1.03807	—	— 8.46	60.06	56.339	—
2.5638	1.09625	—	— 7.22	149.88	57.657	—
5.1420	1.18851	—	— 5.76	300.60	59.568	—

<sup>1)</sup> Exact correspondance between the cohesive force of the inactive gases and of the ions in the solution is, from the foregoing hardly to be expected, though the variation of  $k$  in Fig. 10 and Fig. 12—13 should present similarities. The similarity between the variation from K to Cs and from Cl to J on the other hand, should be more direct.

<sup>2)</sup> Journ. of Am. Chem. Soc. **38**, 70 (1916).

c	d <sub>s</sub>	d <sub>w</sub>	Contract. cm <sup>3</sup>	c · M	a	a Water
Table 55. KCl.						
0.3342	1.01271	0.99707	- 9.68	24.92	55.496	55.343
0.6689	1.02797	—	- 9.07	49.87	55.628	—
1.6694	1.07243	—	- 8.02	124.47	55.955	—
3.3481	1.14391	—	- 6.72	249.63	56.344	—
Table 56. RbCl.						
0.4026	1.03232	—	- 9.76	48.68	55.404	—
0.8089	1.06759	—	- 9.39	97.80	55.447	—
2.0185	1.17076	—	- 8.24	244.06	55.475	—
4.0487	1.33989	—	- 6.87	489.53	55.298	—
Table 57. CsCl.						
0.4278	1.05174	—	- 1.74	71.99	55.238	—
0.8601	1.10654	—	- 1.23	144.73	55.106	—
2.1465	1.26769	—	- 0.02	361.19	54.610	—
4.3051	1.53400	—	+ 1.34	724.42	53.547	—
Table 58. KBr.						
0.4573	1.03554	—	- 8.29	54.43	55.371	—
0.9194	1.07386	—	- 7.70	109.43	55.370	—
2.2944	1.18625	—	- 6.62	273.08	55.274	—
4.6017	1.37090	—	- 5.40	547.69	54.897	—
Table 59. KJ.						
0.5532	1.06328	—	- 6.65	91.84	55.027	—
1.1123	1.12962	—	- 6.16	184.66	54.675	—
2.7758	1.32516	—	- 5.20	460.84	53.527	—
5.5672	1.64833	—	- 3.98	924.27	51.324	—

By graphic reading I have determined a by c = 1 for the various salts and thence calculated a (c = 1) : a (water),

Salt	a (c = 1)	$\frac{a (c = 1)}{a (Water)}$
Li Cl . . . . .	56.34	1.018
Li Cl, H <sub>2</sub> O . . .	55.34	1.000
Na Cl . . . . .	56.30	1.017
K Cl . . . . .	55.74	1.007
Rb Cl . . . . .	55.48	1.003
Cs Cl . . . . .	55.06	0.995
K Br . . . . .	55.36	1.000
K J . . . . .	54.75	0.989

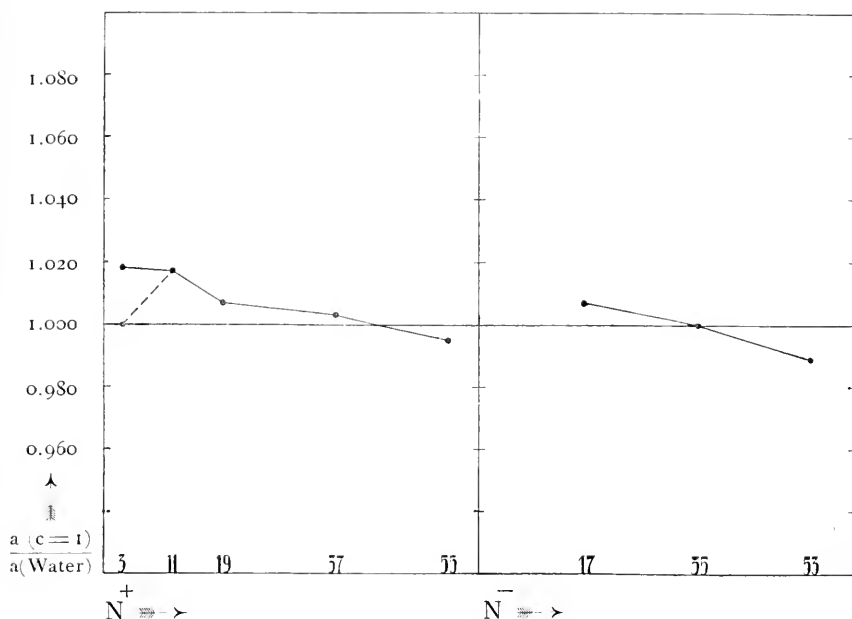


and to give a clearer view I have in Fig. 11 set off this as ordinate, the abscissa being, in the case of the potassium salts, the atomic number of the anion, in the case of the chlorides, that of the kation.

Fig. 11.

Alkali chlorides.

Potassium halogenides.



The dotted line shows how the curve would run if we suppose the Li-ion hydrated with one water molecule.

The essential point, as these curves show, is that this effect also runs parallel when we vary the anion and when we vary the kation. They also reveal, as does the curve for compressibility, pointed out by Geffchen (*vide infra*) a fine similarity to the corresponding curves for the salting out effect on the gases and aniline and ethyl ether. As the influence of molecular density on the solubility must probably depend on how near the molecules of the substance in question can approach to the water molecules, it is possible that this effect, which in the case of substances slightly soluble would no doubt play an essential part, will in the case of substances which can approach very nearly to the water molecules, yield to the forces of cohesion. I must emphasise the hypothetical character of these suggestions, and

point out that we have no means at the moment of measuring the absolute value of the influence of molecular density upon solubility from the molecular-kinetic point of view. It seems to me however, that the proportion  $a$  ( $c = 1$ ) :  $a$  (water) is so little different from 1 that it is possible that this effect may not have the predominant effect with the salts referred to which has been ascribed to it.

But to arrive at a real elucidation of this question demands far more systematic experimental work directed towards this problem than the present. Among other things, it is not enough to systematise the selection of salts; the neutral components must also be chosen with a definite aim in view, i. e. in one of the organic series. Not only the solubility must be measured, but all such physical qualities, both of the pure materials and of the mixtures which might be considered of importance to the question. Until this is done, I do not think the problem (and the question of the salting out effect generally) is ripe for thorough treatment, and at the moment we are in essential points forced to be content with conjecture.

### *Summary.*

I have throughout the foregoing endeavoured to set forth the most important causes of salting out. The suppositions on which my observations are based are in the highest degree simplifying, so that we can only expect a very rough agreement with the experiments. The result is as follows:

In a fluid phase with 3 components, water, salt and the neutral substance  $S$ , which is supposed to be present at a slight concentration, an alteration in activity of  $S$  is determined partly by the dislocation of concentration between salt and water, partly by the total alteration in the density of the phase. The effect of the latter is not amenable to closer investigation; the influence of the former, however, can under certain circumstances be qualitatively determined.

The nature of the particles whose mutual forces we are investigating, is as follows: a) ions, charged particles which are at the same time quadrupoles, b) water molecules, dipoles, simultaneously quadrupoles, and c) molecules of  $S$ , quadrupoles (possibly dipoles). The potential of a molecule is determined through

the polarisation<sup>1)</sup> of its atomic system, caused by the electric field from other particles (Debye) and both polarisation and the electric force are determined by the structure of the particles. While in the foregoing I have but lightly touched upon the field of the charges and the dipoles, I have used some calculations of Debye's concerning the mutual forces of the quadrupoles, which must be supposed to answer to the ordinary cohesive forces of van d. Waals, for elucidating the contents of, and variation with the nature of the ions in the constant  $k$  in the equation

$$\log f = k \cdot c.$$

The main result of these considerations is that owing to the relations existing between  $b$  and  $a$  in van d. Waals' equation, as shown by calculation of the activity coefficient in gases,  $k$  will, as a result of this action, vary from ion to ion and in such a manner that it will fall with increasing size and polarisability of the ion (Fig. 10) and that the variation from KCl to CsCl will run parallel with that from KCl to KJ, this being more or less reproduced in curves such as those in Fig. 10 (the normal curve, see p. 42). This parallelism can be effaced by the influence of the chemical affinity between the ions and S, the sign of the ionic charges being determinative for this. The question as to influence of association of the water (or its alteration with the concentration of the salt) and the hydration of S we have postponed to a later section, together with the specific action of the ionic charges.

While the variation of the share of the molecular forces in  $k$  is determined by the interaction between ion and S alone, and rises with the magnitude and polarisability of S, the field of attraction of the water also enters into the absolute value of  $k$ , whereby, owing to the great solvent power of the water,  $k$ , which for gases is chiefly negative, will in solutions be chiefly positive, answering to an increased activity of S. The field of the water does not enter into the variation of  $k$ , however, save through the alteration in the molecular density.

These quadrupole calculations are one-sided, as no account is taken of the field of the ionic charges, and a possible dipolar

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<sup>1)</sup> "The deformation" of the electron system in the ions, as Fajans has called it, is really nothing but Debye's polarisation.

structure of S; it is to be hoped that later works will take these points into consideration as well. I do not think, however, that these conditions will affect the result in qualitative respects. In a system of molecules whose relative distances are of the same order of magnitude, or even less indeed than the molecules themselves, the definition of an ion or a dipole relative to a quadrupole is an extremely difficult matter, and a thorough treatment of this almost continuous system of linked atoms and ions almost equally so. But from these complex conditions, the quantity P, the refraction of the molecules or ions, stands out as a main factor. We shall see later on how a similar quantity plays a great part in determination of the influence of chemical affinity.

Connected with the treatment of this complicated system is the question as to scope of validity for van d. Waals' equation. As it is derived as a first approximation, equations (1) and (2) are really equally justifiable. (1) however, as we shall see agrees best, but we have no means on the present basis of ascertaining why.

Similarly, we find it impossible, from van d. Waals' equation, to say anything as to the dependence on temperature of  $f$  in solutions or the relation between  $U$  and  $f$ . (A line taken by Mc. Keown<sup>1</sup>) in dealing with some experiments by Thorne<sup>2</sup>) on the salting out of ether with sodium chloride, and where he arrives thermodynamically at the expression:

$$RT \frac{d \ln f}{dc} = \frac{dU}{dc}$$

seems to me far from sound. It does not hold good in any of the causes I have investigated, but only for ether, which is doubtless accidental).

## 2. The normal Curve.

### *Experimental Material.*

As mentioned in the introduction, there is an abundance of literature on the problem of salting out effect. Thus hydrogen has been investigated by Steiner<sup>3</sup>), Braun<sup>4</sup>) and Knopp<sup>5</sup>);

<sup>1</sup>) Journ. of Amer. Chem. Soc. **44** (Juni) (1922).

<sup>2</sup>) Journ. of Chem. Soc. **119**, 262 (1921).

<sup>3</sup>) Wied. Ann. **52**, 275 (1894).

<sup>4</sup>) Zeitschr. f. phys. Chem. **33**, 721 (1900).

<sup>5</sup>) — — — — **48**, 97 (1904).

carbondioxide by Setschenow<sup>1)2)</sup> and Geffchen<sup>3)</sup>; nitrous oxide by Geffchen(l. c), Knopp (l. c.), Roth<sup>4)</sup> and Gordon<sup>5)</sup>; nitrogen by Braun (l. c.); ether and aniline by Euler<sup>6)7)</sup>; ether by Torin<sup>8)</sup> and Thorne (l. c.); phenyl tiocarbamide by Rothmund<sup>9)10)11)</sup> and Biltz<sup>12)</sup>; salicylic acid, benzoic acid and o-nitrobenzoic acid by Hoffmann and Langbeck<sup>13)</sup> and o-phthalic acid by Rivett and Rosenblum<sup>14)</sup>; boracic acid by Bogdan (l. c.). I have made an extract from these investigations, calculating k for the neutral substances and salts given in Table 61 and 62

Table 61. Values of k.

Salts = Substances in- vestigated:	Li Cl	Na Cl	K Cl	Rb Cl	Cs Cl	Authority
H <sub>2</sub> . . . . .	0.076	0.108	0.094	—	—	Steiner
CO <sub>2</sub> . . . . .	0.074	0.091	0.078	0.067	0.053	{ Geffchen Setschenow
N <sub>2</sub> O . . . . .	0.091	0.109	0.101	0.093	0.077	{ Geffchen Gordon
Ethyl-ether . . .	0.176	0.238	0.220	—	—	{ Euler Thorne
Aniline . . . . .	0.110	0.150	0.130	—	—	Euler
o-phthalic acid abt. . . . .	0.166	0.128	0.055	0.030—0.020	0.020	{ Rivett Rosenblum.

Table 62. Values of k.

Salts = Substances in- vestigated:	K Cl	K Br	K J	Authority
CO <sub>2</sub> . . . . .	0.078	0.070	0.065	Geffchen
N <sub>2</sub> O . . . . .	0.101	0.095	0.090	Geffchen
o-phthalic acid abt. . . . .	0.055	0.034	0.016	{ Rivett Rosenblum.

<sup>1)</sup> Zeitschr. f. phys. Chem. **4**, 117 (1889).<sup>2)</sup> Ann. de Chim. et de Phys. (6) **25** (1892).<sup>3)</sup> Zeitschr. f. phys. Chem. **49**, 257 (1904).<sup>4)</sup> — . . . — **24**, 114 (1897).<sup>5)</sup> — . . . — **18**, 1 (1895).<sup>6)</sup> — . . . — **31**, 360 (1899).<sup>7)</sup> — . . . — **49**, 303 (1904).<sup>8)</sup> — . . . — **89**, 685 (1915).<sup>9)</sup> — . . . — **33**, 401 (1900).<sup>10)</sup> — . . . — **69**, 533 (1910).<sup>11)</sup> Zeitschr. f. Elektrochem. **14**, 532 (1908).<sup>12)</sup> Zeitschr. f. phys. Chem. **43**, 41 (1903).<sup>13)</sup> — . . . — **51**, 385 (1905).<sup>14)</sup> Trans. Far. Soc. **9**, 297 (1914).

As regards the constancy of  $k$  for each salt, i. e. in regard to the validity of (I) I have only found any divergence in the case of o-phthalic acid, and here of such a nature as would be due to that part of the phthalic acid (a rather strong acid) which is dissociated in ions. The values for  $k$  are therefore only approximate values, calculated as  $\log f = k$  for  $c = 1^1$ ). For purposes of comparison, I have shown in tables 63—66  $k$  for all the four substances here subjected to investigation.

Table 63.  $k$  for hydroquinone.  $18^\circ$ .

Anions =	$\text{Cl}^-$	$\text{Br}^-$	$\text{J}^-$	$\frac{1}{2} \text{SO}_4^{--}$
Kations				
$\text{H}^+$ .....	0.108	—	—	0.089
$\text{Li}^+$ .....	0.160	—	—	—
$\text{Na}^+$ .....	0.157	—	—	0.156
$\text{K}^+$ .....	0.113	0.107	0.095	—
$\text{Rb}^+$ .....	0.078	—	—	—
$\text{Cs}^+$ .....	0.014	—	—	—
$\frac{1}{2} \text{Mg}^{++}$ .....	0.149	—	—	0.146
$\frac{1}{2} \text{Ca}^{++}$ .....	0.148	—	—	—
$\frac{1}{2} \text{Sr}^{++}$ .....	0.150	—	—	—
$\frac{1}{2} \text{Ba}^{++}$ .....	0.147	—	—	—
$\frac{1}{3} \text{Al}^{+++}$ .....	0.162	—	—	—
$\frac{1}{3} \text{La}^{+++}$ .....	0.136	—	—	—

<sup>1</sup> The temperature at which all these solubility determinations were made varies from  $18^\circ$  to  $25^\circ$ . As the alteration of  $k$  with  $T$  is but slight, this feature does not play any considerable part.

Table 64. k for quinone. 18°.

Anions =	$\text{Cl}^-$	$\text{Br}^-$	$\text{J}^-$	$\frac{1}{2} \text{SO}_4^{--}$
Kations				
$\text{H}^+$ .....	abt. - 0.027	—	—	0.027
$\text{Li}^+$ .....	abt. 0.066	—	—	—
$\text{Na}^+$ .....	abt. 0.056	—	—	0.190
$\text{K}^+$ .....	0.023	- 0.040	—	—
$\text{Rb}^+$ .....	0.019	—	—	—
$\text{Cs}^+$ .....	0.010	—	—	—
$\frac{1}{2} \text{Mg}^{++}$ .....	—	—	—	0.179
$\frac{1}{2} \text{Ca}^{++}$ .....	abt. 0.060	—	—	—
$\frac{1}{2} \text{Ba}^{++}$ .....	abt. 0.046	—	—	—

Table 65. k for succinic acid. 18°.

Anions =	$\text{Cl}^-$	$\text{Br}^-$	$\text{J}^-$
Kations			
$\text{Li}^+$ .....	0.146	—	—
$\text{Na}^+$ .....	0.085	—	0.033
$\text{K}^+$ .....	0.018	0.002	- 0.025
$\text{Rb}^+$ .....	- 0.004	—	—
$\text{Cs}^+$ .....	- 0.024	—	—

Table 66. k for boracic acid. 18°.

$\text{H}^+$ .....	0.082	—	—
$\text{Li}^+$ .....	0.076	—	—
$\text{Na}^+$ .....	0.009	—	—
$\text{K}^+$ .....	- 0.027	—	0.000
$\text{Rb}^+$ .....	- 0.036	—	—
$\text{Cs}^+$ .....	- 0.034	—	—
$\frac{1}{2} \text{Mg}^{++}$ .....	0.082	—	—
$\frac{1}{2} \text{Ca}^{++}$ .....	0.071	—	—
$\frac{1}{2} \text{Ba}^{++}$ .....	0.033	—	—

As will be seen from Fig. 1--9 and the corresponding tables,  $k$  is not always a constant. The main divergence falls for quinone in the solutions of the salts  $\text{LiCl}$ ,  $\text{NaCl}$ ,  $\text{CaCl}_2$ , for hydroquinone in solutions of the salts  $\text{RbCl}$  and  $\text{CsCl}$ , where, however, it lies on the verge of experimental error. In the case of succinic acid, the weak ionisation makes itself apparent,  $k$  rising with the concentration of the salt, though only slightly. I have not been able to find any explanation of the divergence in the case of quinone. In the case of  $\text{NaCl}$  and  $\text{CaCl}_2$ , equation (2) seems to agree, and we are therefore, from the foregoing, beyond the possibility of any explanation in the first approximation. I will not therefore go into this question. In cases of divergence, the stated values for  $k$  are approximated comparative quantities, arrived at by taking the mean of the values at all concentrations.

There are still two things which must be pointed out, before proceeding to investigate the variation of  $k$  with the nature of the ions.

We have in the foregoing taken it for granted that the concentration  $s$  of  $S$ , the substance salted out, was so slight that we could disregard the influence on  $f$  of an alteration in  $s$ . In the case of boracic acid, hydroquinone and succinic acid, where the concentrations lie between 0.5 and 1 normal, this is not permissible. An example will explain: In the quinhydrone electrode<sup>1)</sup> the concentration of hydroquinone and quinone is extremely slight (less than 0.02). By potential measurement it is possible

to determine the quantity  $\log \frac{f_H}{f_K}$ ,

where  $H$  and  $K$  refer to hydroquinone and quinone respectively. (See "Sur l'erreur de sel inhérente à l'électrode de quinhydrone" l. c.). In a 4n  $\text{NaCl}$  solution, it is:

$$(0.7048 - 0.6933) : 0.02885 = 0.3986,$$

from which we get  $k_H - k_K = 0.3986 : 4 = 0.0997$ . (By 18°).

The corresponding value of the solubility measurements, then, at which the quinone and to an even higher degree the hydroquinone are in a concentrated state, is (see p. 6 and 12):

$$k_H - k_K = 0.158 - 0.049 = 0.109.$$

Presuming that  $k_K$  coincides in the two methods of measurement, we then find  $k_H$  too high in the latter case, though it

<sup>1)</sup> Cf. E. Biilmann: Ann. de Chimie, 9 s, 15, p. 111 (1921).



must be pointed out that the discrepancy is only abt. twice the equivalent of the experimental error in the potential measurement. We suppose it is due to the decrease in concentration of hydroquinone effected by the addition of salt:

$$0.5103 - 0.1199 = 0.3904 \text{ mol.}$$

The corresponding alteration (increase) of  $\log f$  is:

$$4 \cdot 0.009 = 0.036$$

and  $k$  for the effect of hydroquinone on hydroquinone therefore:

$$-0.036 : 0.3904 = -0.09.$$

If  $U_0$  for hydroquinone be independent of temperature, this energy will be approximately a measure of the potential alteration of a molecule of hydroquinone passing from a point surrounded by hydroquinone molecules to a point surrounded by water molecules. Owing to the negative sign of  $U_0$  there is therefore nothing remarkable in the fact that  $k$ , which is a measure of the effect when we replace water molecules by hydroquinone molecules, should be negative; in other words, that hydroquinone increases the solubility of hydroquinone. The true magnitude of  $k$  is of course too inaccurately determined for us to draw conclusions; it shows the order of magnitude of this "neutral action" of the salted out substance itself on the determination of the salting out effect by means of solubility measurements, an effect which we must bear in mind, but which on the whole will only slightly affect the values for  $k$  given in tables 63 to 66.

The other point I wished to emphasise is the additivity of these ionic effects. It is connected with the supposition as to complete dissociation of strong electrolytes, and has been tacitly assumed as the basis of the foregoing. Experiments seem to justify this, but it must be pointed out that the ionic effect on density of salt solutions for instance is not additive. Nevertheless I will, in the following, for instance in the case of hydroquinone and  $K_2SO_4$ , quinone and  $MgCl_2$  avail myself of the supposition as to additivity in order to determine  $k$ , where no direct measurements are found.

Fig. 12.

Alkali chlorides.

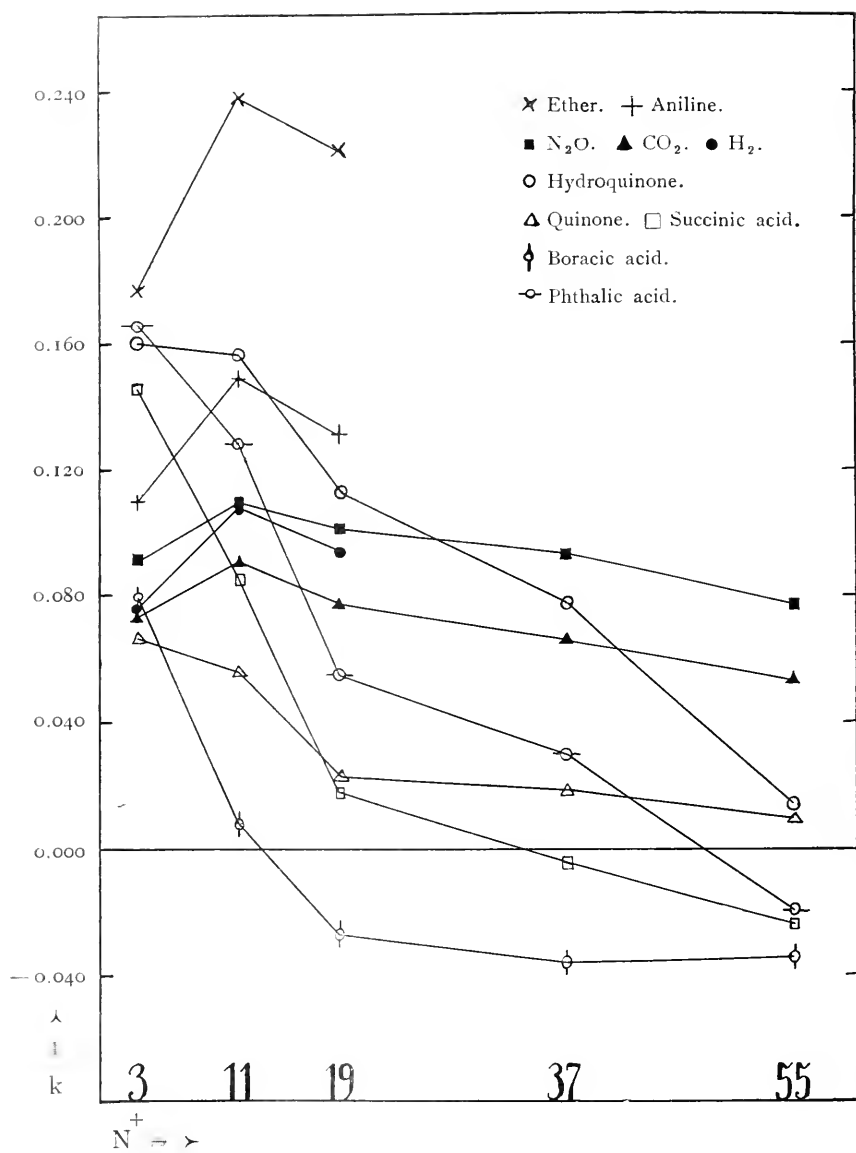
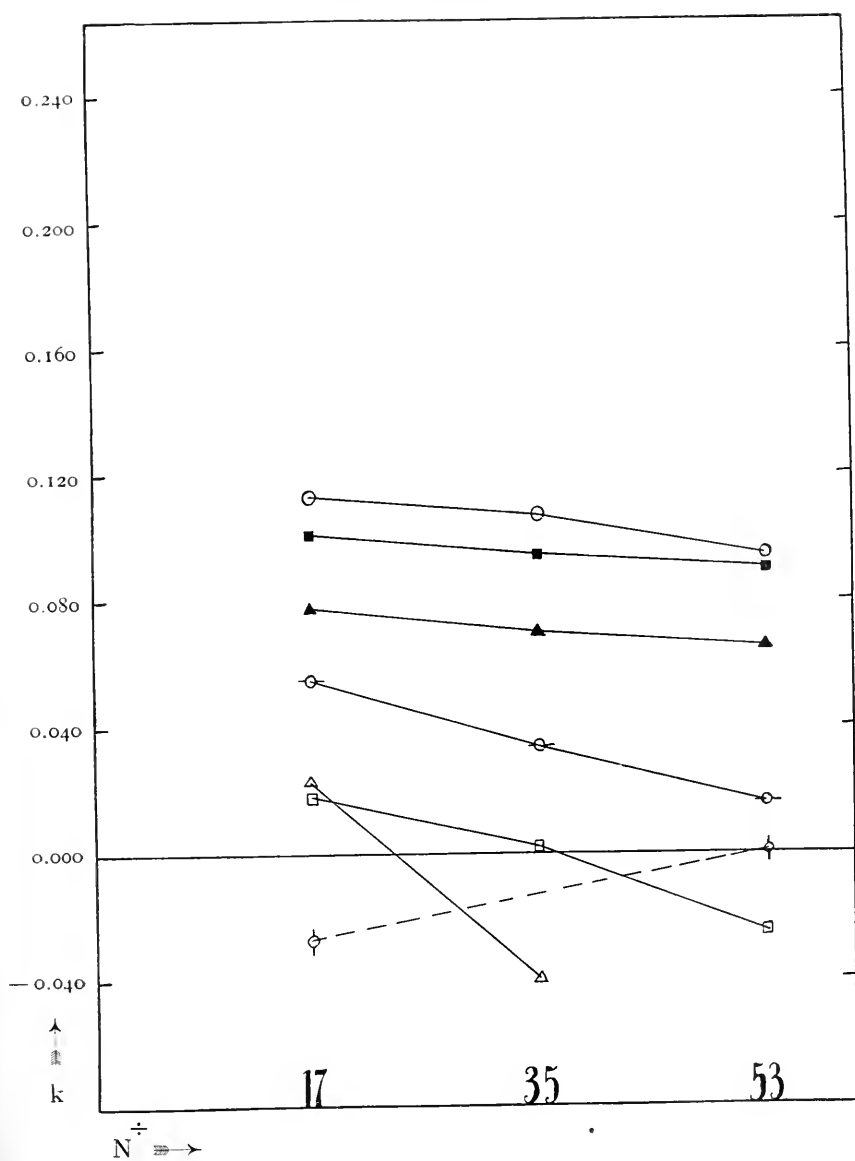


Fig. 13.

Potassium halogenides.



*The Curves for k.*

In Figs. 12 and 13 will be found graphs for  $k$  in its relation with the alkali halogenides. In both figures,  $k$  is the ordinate; in Fig. 12, which comprises the alkali chlorides, the atomic number of the kation is the abscissa, in Fig. 13, showing  $k$  for potassium halogenides, the atomic number of the anion is used as abscissa. The figures thus correspond exactly to Figs. 10 and 11, and as was to be expected, the curves on the right side of the sodium in Fig. 12 present a marked resemblance to the curves in Fig. 13 and with the curves in Figs. 10 and 11. According to the definition of the normal curve (p. 42) the curves for  $\text{CO}_2$  and  $\text{N}_2\text{O}$ , (which by the way resemble each other in various ways) succinic acid and phthalic acid, have a normal course in the main, while hydroquinone and quinone with boracic acid in a lesser degree, have abnormal curves. The direction of the curve for boracic acid is also divergent, rising from  $\text{RbCl}$  to  $\text{CsCl}$  and from  $\text{KCl}$  to  $\text{KJ}$ , which, however, as far as I can see, has its natural cause in the chemical constitution of the boracic acid, to which I shall presently return, together with the question of hydroquinone and quinone.

As regards that part of the curves in Fig. 12 which lies on the left side of  $\text{KCl}$ , the gases, aniline and ether follow, as already mentioned, the curves for molecular density, while the others seem rather to follow Fig. 10 though this is rendered somewhat uncertain by the lack of  $k$  for neon, and the generally more complex conditions here.

There are not a few previous theoretical works on this subject. for the most part the same as those quoted on p. 48—49. The hypotheses of principal importance here are the hydration hypothesis and the hypothesis of alteration in internal pressure (Euler, Geffchen). To the former I shall presently refer; the latter is closely connected with the consideration of alteration in molecular density, as the quantities by means of which we estimate internal pressure: surface tension, compressibility, vary parallel with the molecular density (see Geffchen l. c.). The contraction on solution of salt in water on the other hand, is a combined action of several factors, and cannot therefore, as has incorrectly been done (Euler) be taken as a measure of the alteration in internal pressure. I have chosen the molecular density because

it stands in a certain natural relation to the other views here advanced; it tells neither more nor less than for instance the compressibility.

### 3. The Anormal Curve.

#### *Hydration.*

In the earlier part of this work we have as far as possible disregarded the chemical compounds — considering that all which concerned them belonged to the special part of the problem. Hydration, however, which comes under this head, has been widely used as an explanation of the salting out effect. That I have nevertheless postponed the treatment of this to the present special section, is due to the fact that the commonest view regarding it is marked by a certain lack of lucidity which renders it doubtful.

The simplest view which has made itself apparent is just this: that a gram-molecule of the substance S, in order to become dissolved to the point of saturation, requires exactly as many water molecules as the saturated solution contains per gram-molecule. When we dissolve salt in this, the salt takes water and combines with it, so that the solubility of S decreases in proportion to the reduced quantity of available water. The lack of clearness in this mode of view lies in the difficulty of conceiving what is meant by S requiring such and such a quantity of water to dissolve it, when it does not combine with it, which we cannot possibly imagine to be the case with a substance slightly soluble; moreover, this view leads to too high and highly variable hydration figures for the salt (abt. 20 to 30 water molecules per ion) and must therefore doubtless be regarded as incorrect.

A thermodynamic expression for the influence of hydration in the supposition that S is itself hydrated, in other words, that the action of the water molecules on S must become apparent in a chemical combination, is obtained in the following manner. (See "On the activity coefficient for ions" etc. l. c.). If S be bound to  $m$  water, the equilibrium equation for the transition from hydrate to non-hydrate is:

$$a_{iH} \cdot a_{H_2O}^m = k' a_H \quad (H \text{ hydrate, } iH \text{ non-hydrate})$$

or

$$a_H = k'' \cdot a_{iH} \cdot \left(\frac{p}{p_0}\right)^m,$$

$p$  being the vapour pressure of the water in the salt solution,  $p_0$  the vapour pressure of pure water. Supposing the activity coefficient of the hydrate to be 1, and that the quantity of non-hydrate is small in comparison with that of hydrate, and that the solution is saturated with  $S$ , so that  $a_{iH} = \text{constant}$ , then:

$$s_a = k''' \cdot \left(\frac{p}{p_0}\right)^m \quad \text{and} \quad \frac{S_0}{S_a} = \left(\frac{p_0}{p}\right)^m,$$

from which

$$\log f = m \cdot \log \left(\frac{p_0}{p}\right).$$

Now as mentioned earlier, in the case of many salts, including the alkalihalogenides, the following holds good:

$$\log \left(\frac{p_0}{p}\right) = k_1 \cdot c,$$

where the constant  $k_1$  for the various salts only varies very slightly about the value 0.016. We therefore get:

$$\log f = 0.016 \cdot m \cdot c.$$

If we regard the hydration of  $S$  as the only cause of salting out, then, in order to explain the great variation of  $k = k_1 \cdot m$  with the nature of the salt, owing to the slight variation of  $k_1$  from one salt to another, we must suppose that  $m$  is very great, whereby  $k$ , in the absolute, attains an extremely high value. We are further forced to suppose that  $m$  in many cases must be different in solutions of the different salts, which of course cannot be the case. The view of hydration as the main cause of the salting out effect must therefore doubtless be abandoned; but that it plays a great part in the absolute value of  $k$ , and also as mentioned (p. 42) in the determination of attractive and repulsive forces is beyond all doubt.

As will be seen, this thermodynamical mode of view of hydration is based on the supposition that  $m$  is independent of the salt concentration, which (as Bjerrum has pointed out) is approximately correct. What applies in the case of hydration, however, applies also in that of association; we can reckon that the degree of association of the water will not depend in any essential degree on the salt concentration. And thus it becomes of less importance to the problem here discussed.

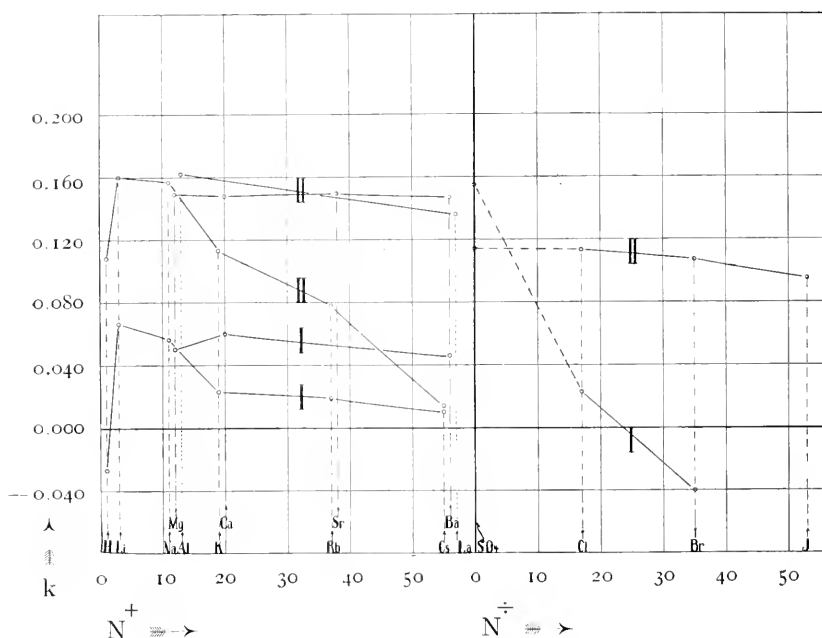
*Chemical "Polarisation".*

We have hitherto disregarded influences of a chemical nature between the ions and S. If we wish to examine the general sides of a problem such as this, it is natural to do so, but if it is desired to explain the deviations from a rule, one turns involuntarily to the old resource: something happens, a chemical reaction takes place. As far as I can see, however, it is not absolutely necessary that the reaction should actually take place; the mere possibility of a reaction is enough to bring about an attraction between two particles. I will endeavour to illustrate this by an example. Quinone is an oxidising substance, which by combining with a molecule of hydrogen becomes hydroquinone. Electrochemically, this is expressed by saying that the quinone has a tendency to catch one or more correctly two electrons, for instance from the platinum plate in the kinhydrone electrode. On catching two hydrogen ions, the hydroquinone is then completely formed. Let us now imagine that we have brought an electron over to the vicinity of a quinone molecule. We know that when the electrons are set in their places, a rearrangement takes place in the quinone molecule; either a genuine benzene ring is formed or a bond between two oxygen atoms ruptures, according to the manner in which we regard the constitution of the quinone. What happens now, from the moment the electron is at a suitable distance from the quinone molecule, until it is finally in its place? We can say that the electron and the quinone molecule during this time form a reaction complex which may be compared to a tense spring, which is released when the reaction takes place. We must imagine a very powerful "polarisation", a displacement in the direction of affinity so to speak, within the quinone molecule, which must make itself apparent in a greatly increased attraction between them, according to the views on the nature of attracting forces on which the foregoing is based. What happens in the case of the electrons however, we must also imagine as taking place with any negative ion, which can approach sufficiently near to affect the quinone molecule, and at the same time we must suppose that the latter will resist the approach of a positive ion, or at any rate, will not be more affected by it than might be expected from its structure and the polarisability of quinone

generally; in a word, such as falls within the line of the normal curve.

Since however, as already noted, an increased polarisability of the affected molecule increases the steepness of the curve for salting out in the highest degree, we must expect, in the case of quinone, to find a considerable difference between the slope

Fig. 14.  
Chlorides. Potassium salts.

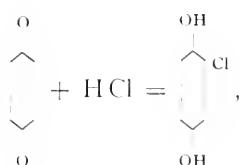


of the curve when the kation is varied and that of the same when the anion is varied; the slope being greater in the latter case. Fig. 14, where  $k$  for hydroquinone and quinone is taken separately and shown graphically as before, shows this. At the same time it will be seen that the  $\text{SO}_4$ -ion<sup>1)</sup>, which we must suppose to be hydrated, has a very high  $k$ , answering to a very low attraction.

Here, however, there is something we must note. As Dr. V. K. La Mer has pointed out to me, quinone in HCl forms chloro-hydroquinone as

<sup>1)</sup> The  $\text{SO}_4$ -ion I have arbitrarily given the atomic-number 0.

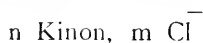




which is really the basis for production of this substance. This process is doubtless accountable for the great fall in  $k$  from the Li ion to the hydrogen ion at quinone. It is further likely that it is the affinity in this process which is the cause of the increased attraction from the chlorine to the bromine ion, which also throws new light on the slight effect of the sulphate ion.

That the process should really take place in solutions of sodium chloride for instance, it is difficult to believe. This would mean, for instance, that in 0.01  $n$  HCl + 1  $n$  NaCl, a great part of the quinone would be transformed into chlorohydroquinone, which would occasion a considerable alteration in the concentration of hydrogen ions. This however, is not the case, as is shown by the agreement between measurements of solubility and measurements with the quinhydrone electrode, where the concentration of hydrogen ions forms a part.

Two other points further argue against chemical compound. If a complex of the following indefinite character:



be formed, then there would have to be a factor  $f_{\text{ion}}$  entering into the activity coefficient for quinone, owing to the charging of the complex, this factor being determined by

$$\log f_{\text{ion}} = -k \cdot c^x$$

where  $x$  is less than 1 ( $1/2$ — $1/3$ ). This would give a  $k$  increasing with the salt concentration (as with phthalic acid and succinic acid) whereas in reality, the opposite is the case.

Our view is likewise supported by a colorimetric determination proposed to me by Prof. Bjerrum, for which I here beg to express my thanks; with salt solutions concentrated up to 4 normal there was found no alteration in shade or intensity of colour of the quinone.

With hydroquinone, the position should be the reverse of that for quinone. Hydroquinone is a reducing substance, and should therefore preferably be polarised, attracted, by positive

ions, which its curves also show to be the case. It is also evident that the hydration which must be supposed to be prominent in the case of the divalent and trivalent ions, completely covers this effect.

Finally, with regard to boracic acid, boron and nitrogen are as known placed on both sides of carbon in the periodic system. Just as ammonia  $\text{NH}_3$  catches a hydrogen ion and forms an ammonium ion, so also we can imagine that boracic acid  $\text{B(OH)}_3$  would have a tendency to complete itself with a hydroxyl group, as the four hydroxyl groups arrange themselves in a stable and harmonious configuration. We can also further conceive, as mentioned under hydration, that any negatively charged ion sufficiently small could take the place of a hydroxyl ion, in other words, we may expect to find an affinity to negative ions increasing with the decrease of the atomic number. This is also the case, as we may see from Fig. 13. When a real reaction takes place it is just as hard to decide as in the case of hydration.

The chemical "polarisation" referred to under hydroquinone and quinone I should like to bring forward here as a factor which it may possibly be necessary to consider in other cases where we have to deal with deviations from the gas laws in concentrated solutions. Among other things, it will probably play some part in the attraction exerted by the ions of weak or unstable acids or bases on the ions, positive or negative respectively, in concentrated solutions.

#### 4. Heat of Solution.

On comparing Tables 48 and 49 with 63 and 64, we notice the parallelism between the alteration of  $U_n - U_o$  and  $k$  with the nature of the salt, but at the same time we see how much greater  $U_n - U_o$  is for quinone than for hydroquinone. As previously mentioned, I have not been able to find any connection between the alteration of the heat of solution and the affinity with salt concentration. A factor which I have not mentioned before, and which plays a contributory part in these difficulties, is the alteration of the dielectricity constant with the temperature. It exerts a peculiar influence here, into which, however, I shall not enter further at present.

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## C. PRACTICAL APPLICATION.

I will not go very closely into the practical application of measurements of the activity of hydroquinone and quinone. The following observations, however, may perhaps be of use.

In place of the formula given by Biilmann (see Ann. Chemie l. c.) for calculation of  $p_H$  in diluted solutions:

$$p_H = \frac{0.3665 - \pi_k}{0.0577},$$

where  $\pi_k$  is the potential of the following element:

Pt, quinhydrone, experimental liquid/3.5 n KCl/0.1 n KCl, HgCl, Hg (i. e. using 0.1 n calomel electrode) we obtain for concentrated solutions (cf. "Sur l'erreur de sel inhérente à l'électrode de quinhydrone" l. c.):

$$p_H = \frac{0.3665 - \pi_k}{0.0577} - 0.5 \cdot \log \frac{f_1}{f_2},$$

where index 1 and 2 refer to hydroquinone and quinone respectively, and  $f$  is the activity coefficient for these substances in the experimental liquid.

The correction term varies greatly with the concentration of the salt and with its character, as is seen from Tables 63—66:

$$0.5 \cdot \log (f_1/f_2) = 0.5 (k_1 - k_2) \cdot c_n.$$

In 1 n KBr solution, for instance, it is:

$$0.5 (0.107 \div (\div 0.040)) = 0.074 \text{ in } p_H,$$

in 4 n KBr:

$$0.29 \text{ i } p_H,$$

whereas in 1 n  $MgSO_4$  solution it is:

$$0.5 (0.146 - 0.179) = -0.017 \text{ in } p_H.$$

Generally speaking, we can say that the correction will be of no importance in salt solutions where both ions are hydrated. For the rest we must, in the case of the salts here investigated, use the tables and curves in the experimental section, and not the values of  $k$ , as those for quinone are approximated; in other words, we must read from the curves  $\log f_{\text{hydroquinone}}$  and  $\log f_{\text{quinone}}$  at the concentration of salt employed, and find the correction by subtraction.

### Summary.

A. Some investigations are given, dealing with the influence of salts on the solubility of hydroquinone, quinone, succinic acid and boracic acid. The activity coefficient for these substances,  $f$ , is determined for each salt concentration  $c$ , and the following law in the main confirmed:

$$\log f = k \cdot c,$$

where  $k$  is a constant independent of the salt concentration, but dependent on the nature of the four substances, on the nature of the salt, and on temperature. On the basis of determinations of solubility at different temperatures,  $U_0$ , the heat of solution in water (or 0.01  $N$  HCl) is found, and for quinone and hydroquinone also  $U_n$ , the heat of solution in salt solutions of the concentration 1  $N$ .

B. On the basis of Debye's views as to the nature of cohesive forces, an endeavour is made to throw light upon certain causes of the variation in  $k$  with the qualities of the substance salted out and of the ions, the structure of the monovalent ions, alkali ions and halogen ions being brought in relation to the structure of the corresponding inactive gases. It thus becomes probable that  $k$  must decrease with increasing size of the ions forming the salt, and normally parallel from KCl to CsCl and from KCl to KJ. — The total particle density in solutions of these salts is determined subject to certain simplifying suppositions whereby a similar parallelism is shown to exist. — The hydration of ions is touched upon, and the view expressed that none of the ions  $K^+$ ,  $Rb^+$ ,  $Cs^+$ ,  $Cl^-$ ,  $Br^-$ ,  $J^-$  is hydrated. Attention is drawn to the effacing influence on the forces of cohesion involved by hydration of ion or neutral molecule, and at the same time the view that hydration plays the leading part in the salting out process is rejected.

In agreement with deviations from the mentioned parallelism, as far as regards hydroquinone and quinone, it is shown how the reducing qualities of the former, and the oxydising power of the latter, cause an increased attraction of the former to positive ions, and of the latter to negative ions, whereby the importance of chemical affinity for the determination of the activity

in concentrated solutions, even where no chemical reaction takes place, is made clear.

A special interpretation is found for the salting out effect in the case of boracic acid. On the other hand, the connection between alteration in the affinity and the heat of solution with the salt concentration is not made clear.

Certain hints are given for the practical application of solubility determinations to the correction of measurements with the quinhydrone electrode in salt solutions.

*Carlsberg Laboratory, September 1923.*

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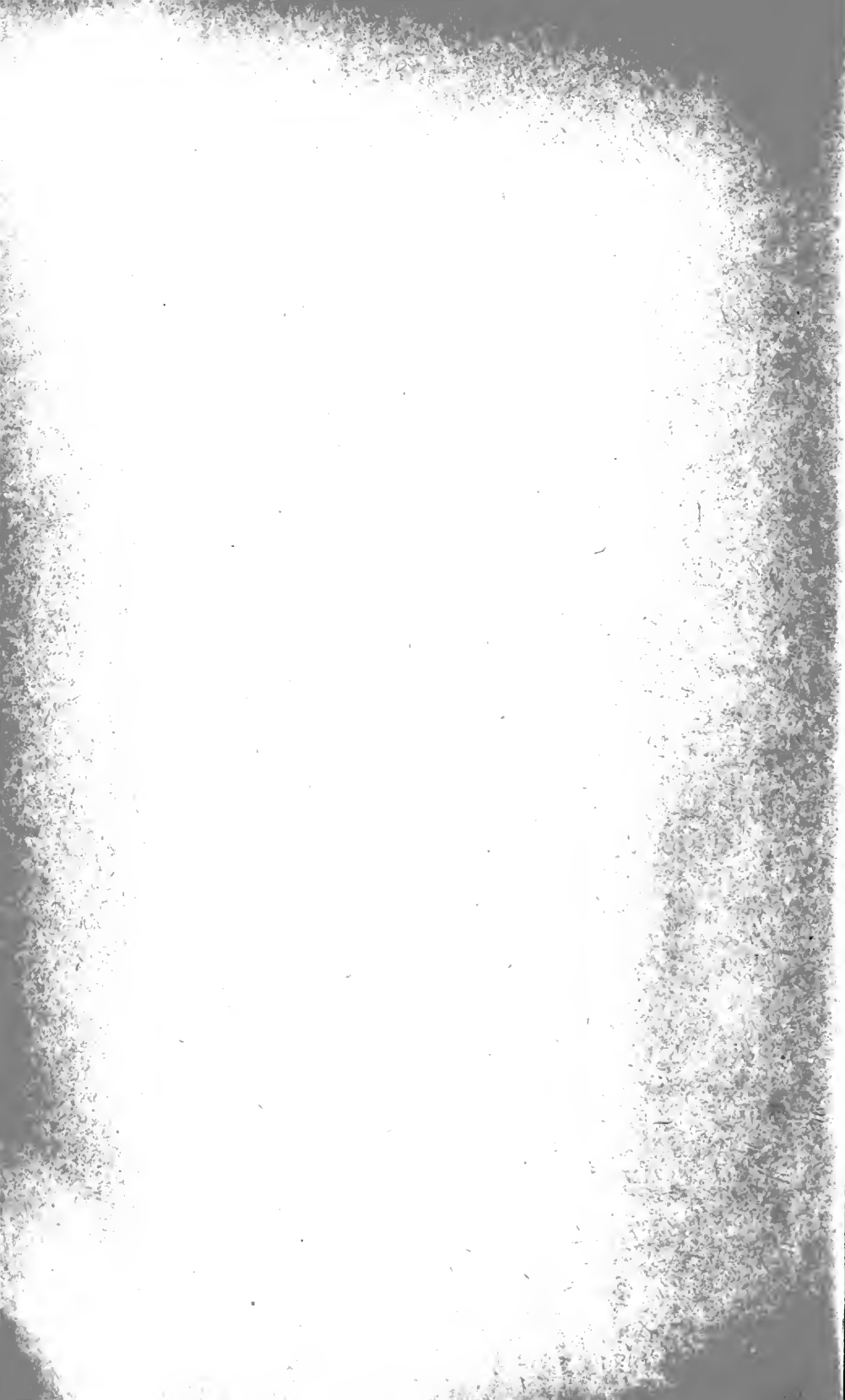
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# ON SEX CHROMOSOMES, SEX DETERMINATION, AND PREPONDERANCE OF FEMALES IN SOME DIOECIOUS PLANTS.

BY

Ö. WINGE.

(WITH 35 FIGURES ON PLATE 1-4.)

**D**uring the years which have elapsed since the beginning of the century, it has gradually been proved that in certain animal organisms, and indeed presumably among all unisexual animals, there exist certain particular sex chromosomes, the distribution of which in the individual determines whether it is to be female or male. As a rule, these sex chromosomes differ from other chromosomes in appearance, and in most cases it has been found that female individuals contain two so-called X-chromosomes in the cell-nuclei, the males on the other hand having an X- and a Y-chromosome. In the formation of the sex cells, then it is always an X-chromosome which enters into the egg, whereas in the sperm cells it is now an X now a Y. If an egg be fertilised with an X-bearing sperm cell, the germ will be female; if the egg be fertilised with a Y-bearing sperm cell, it will be male. Among birds and Lepidoptera as distinguished from other animal organisms, the reverse is the case; here, it is the male which has X + X, the female X + Y. There are, however, many modifications of these conditions; in particular we may find, among certain organisms, the Y-chromosome altogether lacking, so that one sex will have X + X, the other only X, whereby individuals of the one sex will have one chromosome more than those of the other.

In agreement with the facts here stated, sex-linked inheritance has been shown to exist in many cases both among animals and human beings, a type of heredity which can just be

explained by supposing hereditary tendencies, or genes, to be associated with the X-chromosomes. Just as an XY individual will always have received its X-chromosome from an XX individual, so again it will pass it on to an XX individual, thus producing the zig-zag heredity which we call »sex linked«.

That X- and Y-chromosomes however, cannot always be distinguished from each other under the microscope, or even from the remaining chromosomes, is a fact which I have pointed out (1922 a) in the case of the cyprinodont *Lebistes reticulatus*. But that the existence of sex chromosomes can nevertheless be proved is sufficiently evident from the genetic investigations carried out with this little fish. These showed, on the one hand, one-sided masculine inheritance, a mode of inheritance previously unknown, but due to the fact that the factors together with the Y-chromosome, pass from one male generation to the next (Schmidt 1920, Winge 1922 a and b, 1923) and on the other hand sex-linked inheritance (Winge 1922 b, 1923) which must necessarily involve the presence of X-chromosomes. Furthermore, in agreement with the fact that the X- and Y-chromosomes in *Lebistes* are alike in appearance, and can therefore like the other pairs of chromosomes, conjugate prior to sex-cell formation, it was found that a crossing-over of genes took place from the X- to the Y-chromosome and vice versa (Winge 1922 b, 1923) so that the mode of inheritance of a single character fluctuates from sex-linked to one-sided masculine and vice versa.

In the case of plants, it was much longer before chromosome difference was demonstrated in individuals of different sexes, and several investigators have, on the basis of research with various dioecious plants, believed themselves justified in stating that no such difference existed; thus in particular Sykes (1909) and Strasburger (1910). The first instance was shown by Allen (1917, 1919) in the liver moss *Spharocarpos Donnellii*. The investigator here succeeded in showing that female and male individuals differed in the amount of chromosomes, the female plants having 7 autosomes and a voluminous X-chromosome, whereas the males had 7 autosomes and a diminutive Y-chromosome. In the sporophyte, the presence of the large X-chromosome was demonstrated, though Allen did not succeed in finding the Y-chromosome here, this however, being probably only due to the paucity of material. The reduction division of

the sporophyte was likewise not observed. All things considered, however, there can be no doubt but that the sporophyte contains 7 pairs of autosomes + X + Y, i. e. 16 chromosomes in all. — In another species of the same genus, *S. texanus*, Miss Schacke (1919) found corresponding conditions

The first phanerogamous plant in which sex chromosomes were demonstrated is *Elodea Canadensis* (Santos 1923). In the male plant, the only one investigated, the haploid chromosome number 24 was found, though 2 of the 24 pairs observed in reduction division were remarkable in point of size. One of the divergent pairs consisted of two gigantic chromosomes (called L) the other of an unequal pair, in which one partner was diminutive, the other of normal size. This odd pair is doubtless rightly presumed to be the sex chromosome pair, and the smaller partner would probably be the Y-chromosome, the larger X. — Of the 48 chromosomes which the diploid cells must be supposed to contain, the male should then have 44 autosomes (= 22 pairs), 2 large L-chromosomes and an X- a Y-chromosome, so that in reduction division, 22 + L + X would enter into half the pollen grains, and 22 + L + Y into the other half. The somatic cells of the female plant should have 44 autosomes (= 22 pairs, and 2 large L- and 2 X-chromosomes, so that only one sort of eggs would be produced.

The chromosomes in *Elodea Canadensis* then, will appear schematically as follows:

Diploid	Haploid
♂ = 44 + 2 L + X + Y	22 + L + X    22 + L + Y
♀ = 44 + 2 L + X + X	22 + L + X

The sex chromosome pair has been observed both in diakinesis and in the heterotypical metaphase and early anaphase and must, to judge from the description and figures, be considered as sufficiently demonstrated.

In addition to *Sphaerocarpos* and *Elodea*, sex chromosomes have also been found, and that during the present year, in *Rumex acetosa*, in this case by the Japanese investigators Kihara and Ono (1923). — The conditions here found are very peculiar. The diploid chromosome number in the male plant is 15, and in the female 14. Of the 15 chromosomes in the

male plant, 12 are autosomes (6 pairs) while the three last form a group of 1 larger (M) and 2 smaller ( $m_1$  and  $m_2$ ). On reduction division, the two smaller chromosomes are seen attached each to one end of the larger one, the two smaller going always to the same pole, while the large one goes to the opposite. The nuclei of the pollen grains should thus receive either 6 autosomes + 1 sex chromosome (M) — or 6 autosomes + 2 sex chromosomes ( $m_1 + m_2$ ). And as a matter of fact, the homotypical metaphase does show now 7 now 8 chromosomes. The female plant on the other hand has in the somatic cells 6 pairs of autosomes + 2 larger sex chromosomes (M + M) and on reduction division, there should thus always be 6 autosomes and 1 large sex chromosome (M) entering into the eggs. The position for *Rumex acetosa* then can be stated thus:

Diploid	Haploid
♂ = 12 + $m_1$ + M + $m_2$	6 + M    6 + $m_1$ + $m_2$
♀ = 12 + M + M	6 + M

The results arrived at by Kihara and Ono are entirely in agreement with those for *Rumex acetosa* which Correns (1922) obtained in a different way, and which prove that the pollen is decisive in sex determination, so that the male plant must be heterogametic. There is a fine agreement here between the conclusions Correns was able to draw, and the chromosome conditions actually found.

### Own cytological investigations.

Some cytological investigations which I have carried out with dioecious phanerogams have brought to light a new series of instances of the occurrence of sex chromosomes, and I shall in the following pages give a brief account of the conditions found. From these, I have no doubt but that sex chromosomes are found altogether throughout the whole of the vegetable kingdom in dioecious species, though it will not perhaps always be possible to demonstrate the same.

***Humulus lupulus.***

In a previous work on hops (1914) I showed that the diploid chromosome number in both sexes was 20, the haploid 10. The same result was arrived at simultaneously by Tournois (1914) but neither of us had observed the presence of sex chromosomes. Continued investigations however, have shown me that in *Humulus lupulus* there is always a distinct sex chromosome pair, which can be observed on reduction division of the pollen mother cells, both in diakinesis and in the heterotypical division.

Figs. 1 and 2 show two pollen mother cells in diakinesis. The gemini are in early diakinesis always remarkably elongated, often shaped as a figure of eight or circular, but a particular pair of sex chromosomes is always visible, consisting of one shorter and one very long chromosome, attached end to end. The long chromosome is generally constricted at or near the middle, and may thus sometimes suggest the impression of three chromosomes one after the other in a row. At times, the chromosome complex may be curved to a U shape, but as a rule it extends throughout the greater part of the nucleus in contrast to the autosomes. Not all the gemini are drawn in the figures, partly in order to let the sex chromosome pair appear more distinctly, and partly because a number of chromosomes had been cut off by the microtome and appear in a following section. Having, once become aware of the peculiar pair of sex chromosomes in the diakinesis, it is hard to understand how one could have overlooked them before.

When the chromosomes arrange themselves in order to effect the heterotypical division, and especially in the meta-anaphase, the pair of sex chromosome will be very distinct (Fig. 3—5) being remarkable in the considerable difference of size between the two partners. The constriction in the larger one is now rarely visible and in the anaphase, the two odd partners, X and Y, move each to its separate pole. It is characteristic that the small sex chromosome in the meta-anaphase sticks up above the others, but for the rest, the absolute difference in size between sex chromosomes and autosomes is not so great in *H. lupulus* that the sex chromosomes can be recognised with full certainty at later stages than the anaphase. It is a fixed rule that the pair of sex chromosomes lies in the periphery of the nuclear plate.

From this it necessarily follows that half the pollen grains will receive an X-chromosome (presumably the large one) half of them a Y-chromosome, for the homotypical division proceeds quite normally as an equational division, and we can thus assert that in the hop plant, *Humulus lupulus*, the male plant is heterogametic, the female homogametic. The position with regard to chromosomes in the change of generations can be shown as follows:

Diploid	Haploid
♂ = 18 + X + Y	9 + X    9 + Y
♀ = 18 + X + X	9 + X

### ***Humulus Japonicus*.**

My previous investigations with this plant (1914, 1917, 1919) showed that it has, like *H. lupulus*, 20 chromosomes in somatic cells and that the chromosome number is the same for both sexes. In my first publication, I erroneously stated that the species had 8 chromosomes in the haploid phase, but after the appearance of Tournois' work (1914) in which he gave 10. I revised my preparations and found (1917) that his figure was correct.

The two species of *Humulus*, then, not only agree in regard to their chromosome numbers, but also in the matter of sex chromosomes they are altogether similar.

The diakinesis in the pollen mother cells is shown in Fig. 6—9. Just as in its perennial relative, we find here a pair of sex chromosomes lying end to end, and as a rule extending throughout a great portion of the nuclear cavity, even at a late stage of the diakinesis, while the autosomes here are short and fairly regularly arranged in parallel pairs. The large partner of the sex chromosomes is more markedly divided by a median constriction than in *H. lupulus*. indeed, in some few cases, this transverse division may go to such a length that we might with some reason regard the result as a row of three chromosomes together. There is a remarkable similarity between this point and that shown by Kihara and Ono (1923) in the case of *Rumex acetosa*. True, the diakinesis does not appear to be mentioned by these writers in the brief English text (though

possibly in the Japanese) but the statement that *Rumex*, in the heterotypical metaphase, shows a complex of 3 chromosomes, the two smaller ones attached one to either end of the larger one in the middle, and also these writers' Fig. 2, evidently representing the diakinesis of *Rumex*, seem to present a view of conditions much resembling *H. Japonicus*. It is therefore remarkable that *Humulus* shows no suggestion of any such arrangement, with the two exterior chromosome sections going to the same side, the middle one to the opposite pole. Both *Humulus* species have in reality only two sex chromosomes, and it is only the median constriction of the one, which, when especially pronounced, may give the impression that three are present. Fig. 10 shows a single picture of the heterotypical early anaphase, where the sex chromosomes are separated. After separation, the constriction of the larger partner becomes somewhat less marked; indeed, it may often be only discernible from the fact that the chromosome is bent at an angle, the apex of the angle then denoting the point of constriction. As in *H. lupulus*, the sex chromosomes are as a rule separated somewhat earlier than the autosomes.

In *H. Japonicus* also, the male plant is heterogametic and has 9 pairs of autosomes + X + Y, the female having 9 pairs of autosomes + X + X.

In an earlier work (1919) I have pointed out a remarkable feature in the heredity of *H. Japonicus* var. *albomaculata*, a handsome white-mottled variety, occasionally grown in gardens. It was found that the inheritance took place exclusively through the mother, the mottledness or lack of same in the father having no effect at all upon the offspring. The quality was transferred from the mother to all descendants. Neither pure white individuals nor those of a normal green appeared as the offspring of a mottled female plant.

The chromosome conditions which I have now pointed out in *H. Japonicus* have nothing to do with this peculiar inheritance. As in my earlier work, I must presume the explanation to be that the cytoplasm itself is in this case the bearer of the inheritance, and that since the male plant does not transfer the tendency to mottledness to the offspring, no cytoplasm accompanies the male sex nucleus into the egg cell on fertilisation. As we

know, there are other cases of mottledness in which the character must be regarded as not situate in the cytoplasm itself, but in the plastides.

### **Melandrium album.**

This plant has been the subject of close experimental investigation in regard to sex determination and sex-linked inheritance. It is the only plant in which sex-linked inheritance has been shown to exist (Baur 1912, Shull 1914) and we might thus really expect X-chromosomes to be present. As, moreover, these experiments showed that the male plant was the heterogametic, and the female the homogametic sex, it would naturally be of particular interest to investigate the chromosome conditions of this plant.

Not only the mentioned investigations, but also the highly interesting experiments made by Correns with alteration of the sex percentage (1907, 1917) showed that the male plant was here heterogametic. Correns pointed out that by the use of abundant quantities of pollen in the pollination, a considerably greater number of female plants was produced in the offspring than when quite small quantities were used — and many other ingenious modes of interference made it evident that the female-determining pollen was able to send the pollen tube down to the ovules, and effect fertilisation, more rapidly than pollen of male determination. It was only when a small quantity of pollen was used, so as to reduce the competition to a minimum, that the male-determining pollen had a chance of fertilising as many ovules as the female. Altogether, the vital processes in the female determining pollen showed the greater speed, whereby also their vital force was the sooner spent, and in agreement with this it was found possible to show that a relative increase in the percentage of males took place when pollen which had been kept in store was used for the fertilisation.

An investigation of the chromosome conditions in male plants of *Melandrium album* has revealed features much resembling those noted above in the case of *Humulus*, though the sex chromosomes here are even more pronounced. The diploid chromosome number is 24, the haploid 12. It is chiefly the heterotypical and the homotypical division in the pollen mother cells which I have studied.



Fig. 11—13 show the heterotypical meta-anaphase which reveals with unusual distinctness the presence of the sex chromosomes. The larger partner (presumably the X-chromosome) is here gigantic as compared with the autosomes; it is in very many cases somewhat bent to an angle, and shows on occasion precisely the same tendency to median constriction as in *Humulus*. The other partner is hardly much larger than the autosomes, and it is just the difference in dimensions of the sex chromosomes which makes them very conspicuous in reduction division. Fig. 14—15 show the early metaphase of the homotypical division. In Fig. 14, the sex chromosome is distinctly the larger partner, and here, indeed, owing to its peculiar position, appears strikingly enormous. In Fig. 15, which like Fig. 14 shows all 12 chromosomes, it is the smaller sex chromosome which has entered in on reduction division, but its position makes it look somewhat larger in proportion to the autosomes than answers to its actual dimensions.

Schematically, then, the chromosomes in *Melandrium album* appear as follows:

Diploid	Haploid
♂ = 22 + X + Y	11 + X    11 + Y
♀ = 22 + X + X	11 + X

There is thus entire agreement not only between the chromosome conditions of the three species already mentioned, but also between the results of the genetic experiments carried out with *Melandrium album*, and the cytological peculiarities the plant exhibits. As the X-chromosome in all probability is the larger partner in the sex chromosome pair, it is only natural that the female-determining pollen which contains it should have a higher degree of developmental energy than the male-determining, which of course would contain a smaller quantity of chromatin.

*Melandrium rubrum* I have not yet been able to investigate, but it is to be presumed that we shall find here conditions exactly corresponding to those described in the case of *M. album*. True Strasburger (1910) has, after close investigation of the reduction division for the special purpose of observing any unequal distribution of the chromosomes, expressly declared

that no such difference can be shown to exist; but it would rather seem that Strasburger was not thinking particularly of difference in size between the partners in a single pair of gemini, and as *M. rubrum*, like *M. album*, has, according to Strasburger, 11 smaller and one large chromosome in the haploid phase, there is reason to believe that it may prove possible after all to show that the two chromosomes constituting the large pair are not of equal size.

### ***Vallisneria spiralis.***

The three species mentioned in the foregoing belong to the *Lygaeus* type, as far as their sex chromosomes are concerned: here, on the other hand, in *Vallisneria*, we find a different state of things, in which no Y-chromosome occurs. I have only investigated male plants, but as it is in this case also the male which is heterogametic, we can with a high degree of certainty draw conclusions as to the chromosomes of the female plant as well.

The chromosome conditions of *Vallisneria* are not very easy to make clear; the fact that some of the chromosomes at certain stages are fusing together is in particular somewhat disturbing at first, in addition to which, the conditions can only be studied with difficulty during division of the pollen mother cells, as the chromosomes here are markedly lumped together at most stages. It is especially the nuclear divisions in the pollen grains themselves which have elucidated the position, and there are only some few details, hardly essential in this connection, though interesting in themselves, which remain to be cleared up.

The diakinesis in the pollen mother cells gives no distinct picture of the number of gemini. As a rule, 8—10 bodies will be seen (Fig. 16 a and b) in which the duality in some cases distinctly shows them to be gemini; others again appear to be single, and there is as a rule a larger, irregular body present, which does not take any marked colour from Hæmatoxylin. The pollen mother cells are found to a number of abt. 4—5 in each pollen sac and in agreement with this, we find later four times the number of completely formed pollen grains. I have not hitherto been able to trace the process either of the heterotypical or of the homotypical division, for the reasons above mentioned.

Occasionally, however, at the transition to the homotypical metaphase, 9 chromosomes of somewhat different sizes may be distinctly seen, (Fig. 17 a and b) while the metaphase itself, as shown in Fig. 18 may exhibit 8 chromosomes. The last phase of the spireme stage in the young pollen grain likewise fails to give anything definite to go by; as in Fig. 19, we often find abt. 8 chromosomes of different sizes, but there may also be more, and they are then generally shorter (Fig. 20).

Towards the close of the spireme stage, the exine of the pollen grains begins to form, and in the following phases of the division of the primary pollen nucleus, the chromosome conditions of *Vallisneria* appear very clearly especially from the unusual distinctness of the subsequent metaphase. Fig. 21—26 give various nuclear plates at this stage, seen mostly from the pole. Only occasionally do the separate chromosomes here overlap, and it is very plainly evident that there are two kinds of nuclear plates, some with 8 autosomes and a sex chromosome divided by constriction, other having no sex chromosome. Fig. 21 shows an almost ideal nuclear plate, in which the X-chromosome is present, and it will also be found in Figs. 22—23. The X-chromosome is, as will be seen, divided into two, a larger portion, of the same dimensions as the largest autosomes, and a smaller answering, to the smaller autosomes. At a casual glance, then, one may easily arrive at the chromosome number 10 instead of 9 in such plates. But as shown in Figs. 24—26, the X-chromosome is lacking in several nuclear plates, and there is thus no doubt but that the divided X-chromosome in reduction division enters into only half the daughter cells and thus only into half the pollen grains. It is beyond doubt that in *Vallisneria spiralis*, the somatic chromosome equipment of the male plant is  $16 + X$  and that the *Protenor* type has also thus been demonstrated in the vegetable kingdom. As however, the X-chromosome is highly constricted, it is possible that it may at certain stages be divided into two, and that we should therefore find, in somatic nuclei, 18 and 20 chromosomes in the male and female plants respectively.

As regards the sizes of the chromosomes, they can for the mentioned metaphase, be given with great accuracy. Not all the figures 21—26 will give the impression of the constant pronounced difference in size of the chromosomes, as they are in

many cases not situated altogether parallel to the plane of the microscope table. In addition to the X-chromosome, there are 2 long chromosomes ( $4.6\ \mu$ ), 2 of medium length ( $3.4\ \mu$ ) and 4 short ( $2.2\ \mu$ ). The larger section of the X-chromosome is abt.  $4.6\ \mu$ , the smaller abt.  $2.2\ \mu$ . As the pairs are identical, apart from the sex chromosome, in the haploid phase, it would seem that we must regard *Vallisneria spiralis* as didiploid, with the fundamental number 4 for the genus.

The foregoing must be considered sufficient to show that in *Vallisneria* also there are sex chromosomes present, and that the conditions are not the same as described by Santos (1923) in the case of *Elodea*. This despite the fact that both plants are ascribed to the same family the *Hydrocharitaceae*.

Since, however, the following stages show that the chromosomes of *Vallisneria* temporarily fuse to a smaller number, the stages in question will here be briefly described.

Fig. 27 shows a metaphase of the primary pollen nucleus in division, viewed from the side. Fig. 28 shows a plate from the pole, and it is conspicuously evident that the chromosomes here are short, and some already split; some are distinctly of the diplococcus shape, as if two smaller chromosomes had fused together. Fig. 29 shows an early anaphase, side view. Here, it is even more distinctly evident that a fusion of the smaller chromosomes has taken place, though it is not possible to identify the origin of each particular fusion product. Only two large, deeply set chromosomes in the last-named plate are seen not to be fused with others. The late anaphase presents a very remarkable appearance, (Figs. 30—31). As a rule, we here find three very long chromosomes, which, after longitudinal fission, extend from pole to pole. Of these, one has always a large knot in the middle, in the plane of nuclear division, and as this chromosome is evidently always present, it can hardly be the X-chromosome. At the poles, we perceive at the same time some smaller chromosomes, the number varying. The explanation is, that the long chromosomes extending from pole to pole consist partly of fused short ones. Gradually, as the cleft halves of the compound chromosomes reach the poles, they fall to pieces again, and in particular, a smaller chromosome is often seen to be twisted off from polar end of a larger one. — This fact, which is not easy to describe in detail, explains how it is that there

often appear to be only abt. 5 chromosomes present during this division, and it was long before stages were found which showed the true state of things in principle. From Figs. 32 a and b it will be seen that the compound chromosomes in the telophase have again separated, so that 8 or 9 chromosomes will be present at either pole, according, as the X-chromosome is lacking or not.

On the division of the primary pollen nucleus, the spindle of the nuclear division is situated to one side of the cell and one daughter nucleus is thrust right over to the periphery of the cell, separated off by a distinct line from the sister nucleus, so that the latter occupies a central position in the pollen grain, while the nucleus thrust aside has only a small quantity of cytoplasm at its disposal. In my material, one or two small chromosome-like bodies are very often thrust off from the pollen grain nucleus to the cytoplasm of the cell, and these can even take the form of diminutive nuclei themselves. This evidently takes place in the telophase. Both nuclei then go to rest for a comparatively long period, and we therefore very often find the stage at which the pollen grain seems to have a small cell, the primary generative cell, situated like an outgrowth from the main cell. In Fig. 33, both daughter cells are in process of reconstruction after division. Prior to the division of the little generative nucleus, it thrusts itself into the main cell of the pollen grain (Fig. 34), the separating line between the two cells is dissolved, and a nuclear spindle formed, at which about 8 chromosomes were observed (Fig. 35). We thus get two generative nuclei, which are constantly present in the ripe pollen grains. The further biological features of these during germination and fertilisation have been recently thoroughly described by Wylie (1923).

The main points in the chromosome conditions of *Lallisneria spiralis* can thus be schematically stated as follows:

Diploid	Haploid
♂ = 16 + X	8      8 + X
♀ = 16 + X + X	16 + X

### Remarks on other Plant Species.

That it is not always possible cytologically to demonstrate the presence of sex chromosomes in dioecious plants is shown, for instance, by an investigation of *Spinacia oleracea*. I had already previously (1917) mentioned that this plant has 6 chromosomes in the haploid phase, in agreement with what had been shown by Stomps (1910). Though my materiel of *Spinacia* is well suited to a study of chromosomes, inasmuch as I was able to examine a great number of heterotypical anaphases and homotypical divisions in the pollen mother cells, it lacked, however, the heterotypical metaphase and the earliest anaphase, in which the chromosome partners can best be compared. Despite all endeavours, I have not succeeded in demonstrating, from my material up to date, the presence of a pair of sex chromosomes in this plant. The chromosomes, which differ somewhat in size, 3 being somewhat larger and 3 somewhat smaller, are extremely easy to follow. If any difference really exists here, it is so slight, that it would hardly be possible to demonstrate with certainty. Moreover, I cannot doubt but that one of the 6 pairs of chromosomes must be sex determining, in which case, we should have a position answering to that of *Lebistes* in the animal kingdom, where also X and Y are alike, and of the same appearance as the autosomes, and can consequently show crossing-over. — There is, however, one possibility remaining, which should not be overlooked, namely that in this plant, it is the female individuals which are heterogametic. This eventuality will have to be investigated later.

On *Polemonium coeruleum*, a species of which I have examined some material from Ostenfeld's experiments (1923) and where, it is true, dioecy is not found, but where the plants are either females or bisexual, we might a priori imagine the position to be that female individuals were recessive and homogametic (XX), bisexual individuals heterogametic (XY) and that the difference between this plant and the dioecious was only that the male-determining element did not dominate the female, so that X and Y would be able to assert themselves both at once. YY individuals would then presumably perish. This supposition, however, will hardly hold good; the matter appears to be more complex.

*Polemonium coerulcum* has 9 chromosomes in the haploid phase, of which 4 to 5 are elongated during reduction division, the remainder being short; the chromosomes are large, and well suited for closer observation. In some bisexual specimens examined, however, no demonstrable sex chromosome pair was found during reduction division. The chromosomes distribute themselves quite uniformly among the daughter cells.

The sex determination in this species however, seems also hard to understand, as Ostenfeld's experiments show. We often find plants which are intermediate, i. e. having in addition to the female organs, some few stamens here and there in the flowers. — The cytological conditions are in agreement with this also rather complicated; we find various irregularities in the chromosomes which, however, I will not enter into here.

### Sex chromosomes and sex determination.

Having in mind the fact that sex chromosomes, X- and Y-chromosomes, are widely distributed in the vegetable kingdom, one might be tempted to regard the sex determination in dioecious plants as fully made clear. When the female sex cells are all X bearing, the males now bearing an X now a Y chromosome, we have what is in itself a perfectly satisfactory sex determining cell mechanism, and the same applies to the other modifications of this position as shown.

This, is however, not the whole of the matter; experience shows that it is more complicated. Shull (1911) also is led by other experience to the conclusion that various influences can make themselves felt in sex determination. We must be content with saying that the normal sex determination is made clear by the demonstration of sex-chromosomes.

In the cases hitherto investigated, the male plant has been heterogametic, the female homogametic. Raunkiær's experiments with *Rumex thyrsiflorus* (1918) might seem to suggest the opposite, but the experiments made cannot be taken as final. Kihara and Ono's demonstration of heterogamy in *Rumex acetosa* (1923) and Correns' experiments with the same species (1922) lead us at any rate to presume that in *R. thyrsiflorus* also, the male plant is heterogametic. — Inasmuch as the X-chromosome is a female-determining element, the Y a masculine-

determining, dominant in its effect over that of X, the male plant should hold a latent feminine tendency, the female on the other hand, always lacking the corresponding masculine. In the case of plants where a Y-chromosome, or what answers to the same, is altogether lacking in the male individuals, as with *Vallisneria spiralis*, this supposition however, will not hold good, and various experiences show that it is incorrect or insufficient. — We cannot here enter upon any exhaustive account of all the experiments and observations under this head, but I will content myself with drawing attention to some few of them.

In dioecious species, we often find some few female flowers or female organs on the male plants, and some few male flowers or male organs in the female plants. I have frequently observed hermaphroditic, generally abnormal flowers in male plants of *Cannabis sativa*, *Humulus lupulus* and *H. japonicus*. In the case of the last-named, Figdor (1911) has given a fairly detailed account of various observations under this head. As however, it is generally the male plants that exhibit a tendency to hermaphroditism, it might still be explained as due to incomplete dominance of the male-determining tendency in the Y-chromosome over the female determining in the X.

But male organs may also exceptionally be found in female plants. This too I have observed both in *Humulus* and *Cannabis*. — Well known in particular is the remarkable fact that female plants of *Melandrium*, attacked by *Ustilago violacea* (= *U. antherarum*) form anthers, which are, however, always destroyed by the fungus and filled with its spores, while in unaffected female plants no such anthers occur. Shull has suggested occasionally (1910) that in such instance it might in reality be a case of male plants developing female organs. This is disproved by Strasburger (1910). And my own experience goes to show that the latest-formed flowers in a plant of *M. rubrum* so attacked are not affected by *Ustilago*, as the fungus did not follow the growth of the plant out to its ultimate ramifications. Here, normal female flowers were formed. This summer, I endeavoured to graft a shoot of a normal female plant on to an affected plant, in order to ascertain whether the flowers formed on the shoot would be attacked and developed in the masculine direction. It was found, however, that the fungus did not attack an engrafted shoot of this sort; the latter developed normal, female flowers,



which bore fruit. Evidently then, the hyphæ of the fungus are at their best in the initial cells of the very youngest stalk-tips, and cannot penetrate into older parts. — But the fact that a female plant of *Melandrium* attacked by *Ustilago* should form stamens is in itself enough to show that the female plant also contains some masculine tendency.

In *Mercurialis annua*, some female flowers on male plants and vice versa were observed by Strasburger (1910). Offspring from self-pollination of such individuals were always of the sex to which the individual from which they were derived mainly belonged. Figdor also (1911) obtained only male plants as offspring of single female flowers on male individuals of *Humulus Japonicus*.

But the fact that the homogametic female sex has a tendency to the masculine is after all quite in agreement with what we know from the animal kingdom in the case of *Drosophila* (Bridges 1922). As in this insect we must reckon that not only the sex chromosomes may contain tendencies to male and female organs, but also the autosomes. Unisexual individuals of either sex can in their autosomes be equipped with male and probably female tendencies, but the sex chromosomes are the normal regulating mechanism which as a rule determines the sex.

There is much to be done in cytological-experimental directions with regard to abnormal hermaphroditism in the vegetable kingdom. Not least the investigations with *Drosophila* intersexes suggest what we may expect to find in this respect. — There are frequent cases of hermaphroditism in dioecious plants. Shull's investigations with *Melandrium album*, for instance (1911) furnish information as to their hereditary nature. In *Humulus lupulus* and *Cannabis sativa*, I have several times observed plants which might equally well be called male or female. The tendency to fruit formation, however, seems in them to be very slight. As a rule the pollen or the pollen mother cells also degenerate sooner or later, and the cytological phenomena can be so abnormal that it is difficult to say what are the chromosome conditions. I have previously (1914) given some details as to such hermaphroditic individuals. — It seems most likely I think that the occurrence of hermaphrodites is due to non-disjunction.

### On the Preponderance of Females in some dioecious plants.

In the case of several unisexual plants, it has been shown that as a rule, more female individuals than male are produced, both in a state of nature and with experimental material. Some explanation of this is afforded by Correns' experiments with *Melandrium album* (1917) and *Rumex acetosa* (1922). Owing to competition, the male-determining pollen grains, whose pollen tubes are on the average of slower growth than those of the female-determining, are only able to fertilise a minority of the ovules. Correns (1907), Noll (1907) and Strasburger (1910) had, by the way, already come to the conclusion that it was the pollen which determined the sex of the offspring. — With the demonstration of sex chromosomes in dioecious plants, we have reached a step farther. The X-chromosome is undoubtedly the larger of the two sex chromosomes where any difference could be shown up to now, and it is only natural that the pollen grains bearing this, and thus having a larger supply of chromatin, should have more growth energy and thus more frequently be able to fertilise the ovules.

It is interesting to notice in this connection that in *Spinacia* where no difference could be found between the two daughter-nuclei at the reduction division, there seems to be no preponderance of females. Nohara (1923) found in his experiments 495 females and 496 males.

It is not our business here to discuss all the investigations and explanations which have been made as to the numerical proportion between males and females in the various dioecious species; but as counts have been made with material derived from the experiments of the Carlsberg Laboratory, in which I was assisting Dr. Johs. Schmidt some years ago, I append here the figures which then appeared:

*Cannabis sativa*. — Plants sowed in 1913—1918 from fruit of single plants gave the following numbers of female and male individuals:

♀♀	♂♂
26	10
170	154
22	28
225	114
83	38
170	110
256	148
4	2
0	2
68	69
80	64
73	51
225	105
2	1
2	4
14	11
109	152
21	25
18	14
5	14
26	33
79	56
37	32
In all... ♀♀ 1715	♂♂ 1237 = 41.8% ♂♂

As will be seen, there is quite a considerable difference between the quantity of males in the separate lots of offspring. It should here be noted that in the case of hemp, an average difference was noted at a comparatively early stage between the sizes of male and female individuals, so that the latter, which are larger, will in dense stocks probably overwhelm by competition a number of male individuals, and in the case of the material quoted, it is not impossible that such competition between the plants may in some cases have affected the numerical proportions to a certain degree. Apart from such causes as may lie in the different quantities of pollen employed, and hereditary differences in the male plants used, the mentioned secondary factors doubtless count for something. For the rest, the male plants flower on an

average earlier than the females, so that on the other hand relatively few male plants would be supposed to fall under the heading of »individuals not sex-determined«; the number of these cannot be stated, but it fluctuated quite considerably.

A count which I made this summer showed that in a parcel of hemp on the experimental ground at Virungaard, near Lyngby, 615 females and 469 males were found, making 43.3 % male. — Earlier investigations have commonly shown ab. 46 % males.

*Humulus Japonicus*. — The experiments I have made with this plant were chiefly for the purpose of investigating the inheritance in the case of the *albomaculata* character, and only a small number of plants were sex determined during the years 1912—1915. — The sowing of fruits from single plants gave the following results:

Name of father plant	Offspring		
	♂♂ ?	♀♀	♂♂
♂ 1	10	4	4
♂ 1	1	6	4
♂ 1	8	7	1
♂ 3	7	12	1
♂ 3	0	17	3
♂ 3	11	33	2
♂ 3	6	23	2
♂ 3	11	27	9
♂ 3	21	24	2
♂ 3	21	15	2
♂ a	0	8	6
♂ a	0	6	9
♂ a	0	10	11
♂ a	26	4	1
♂ a	0	18	10
♂ a	1	16	12
♂ b	7	16	9
♂ c	0	40	23
♂ c	0	18	13
<hr/>			
Ialt . . .	130	304	124 = 29.0 % ♂♂
	?	♀♀	♂♂
		++	

Though the small amount of material here available does not permit of far-reaching conclusions, it is nevertheless noteworthy that the offspring of ♂ 3 shows a far smaller number of male plants than the offspring of the other males. There is hardly any reason to suppose that in this material, there was any overwhelming of male plants by competition after sowing, or that individuals of unknown sex, if brought to flower, would have affected the proportion to any essential degree, and there does not seem to be any difference in the flowering time of the two sexes.

*Humulus lupulus*. — The conditions here are of the greatest interest. The individuals which were sex-determined in the years 1912—1913 were all offspring of a male plant, noted as ♂ 3, those determined in 1914—1916 being all from ♂ 4. In the following table, the name of the mother plant is also given.

Name of mother plant	Name of father plant	?	Offspring ♀♀	♂♂
Fuggles 175.....	♂ 3	3	16	5
Grön 199 .....	♂ 3	21	89	7
Golding 36 .....	♂ 3	3	41	0
Spalter mittel 16..	♂ 3	26	93	6
Early Bird 167 ...	♂ 3	5	36	4
Roter Saaz 75....	♂ 3	5	38	1
Saaz 73 .....	♂ 3	25	120	19
Spalter mittel 32..	♂ 3	2	64	24
Spalter mittel 136.	♂ 3	1	45	3
Bayrish spät 9....	♂ 3	5	23	3
Saaz 130 .....	♂ 3	3	45	4
Saaz 2 .....	♂ 3	1	36	4
Saaz 132 .....	♂ 3	4	59	1
Braunschweiger 18.	♂ 3	1	31	5
Golding 44.....	♂ 3	2	26	6
Golding 36.....	♂ 3	3	22 <sup>1)</sup>	1
Golding 93.....	♂ 3	4	49	4
Golding 187.....	♂ 3	2	40	3
Brambling 64 ....	♂ 3	3	29	4
Brambling 40 ....	♂ 3	3	34	3
Early Hobbs 61 ..	♂ 3	2	24	0
Graa 41 .....	♂ 3	6	44	4
Asperup 207 .....	♂ 3	3	13	3
Oregon Cluster 60.	♂ 3	57	251	23
In all...		190	1268	137 = 9.8 % ♂♂
		?	♀♀	♂♂

<sup>1)</sup> and 1 hermaphroditic female.

Name of mother plant	Name of father plant	?	Offspring ♀♀	♂♂
New York Spaulding				
English Cluster 99..	♂ 4	31	84	25
Saaz offspring 982...	♂ 4	15	56	19
Dauba 414.....	♂ 4	6	23	1
Graa 531 .....	♂ 4	13	21	2
K. 805.....	♂ 4	7	75	15
D. 102.....	♂ 4	15	81	27
D. 106.....	♂ 4	10	84	28
D. 107.....	♂ 4	2	14	10
Spalter offspring 1262	♂ 4	0	22	5
		alt. ... 99	460	132 = <b>22.3</b> % ♂♂
		?	♀♀	♂♂

There is here undoubtedly a difference asserting itself between the offspring of the two mentioned male plants. In this case especially it must be supposed that the non-determined individuals would not be able to affect the proportion between number of female and number of males. The sex of the plants was determined already in the first year of life, as they were forced under glass, and it was always the plants in poorest light which failed to flower, i. e. groups of individuals placed in an unfavourable position.

There is then, in the common hop, a very marked preponderance of female individuals in the offspring. How far numerical proportion may be altered by the use of a very small quantity of pollen I do not know, but it would seem natural to suppose that the quantity of pollen here also plays a considerable part, for, as with *Rumex acetosa*, in which Correns (1922) showed a marked alteration by using different quantities of pollen, so also in *Humulus* only one ovule is fertilised for each pair of styles, and the distance which the pollen tube has to grow through the tissue of the female flower is quite considerable. — It is most probable, however, that different hereditary tendencies in the different male plants affect the two sorts of pollen, especially their X- and Y-chromosomes, and thus help to determine the percentage of females and males in the offspring.

The results put forward in the present work, together with the investigations of Santos and Kihara and Ono upon chromosomes in the higher dioecious plants, afford a further opportunity of noting the marked resemblance in cytological conditions between plants and animals. And as the investigations progress, the similarity will doubtless also be extended to other species of plants as well, partly in regard to the cytological side of the question, and also in regard to heredity. Whether female heterogamy also exists in the vegetable kingdom is still uncertain.

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By arrangement with the Director of the Carlsberg Laboratory, Dr. Johs. Schmidt, this paper appears among the publications of the Laboratory, the statistical material regarding number of female and male plants in *Cannabis* and *Humulus* being derived from Dr. Schmidt's experiments, — while the cytological investigations of the *Humulus* species also form a natural supplement to my previous results published in the papers issued by the Laboratory. The cytological work, however, was carried out at the Genetic Laboratory of the Royal Veterinary and Agricultural College.

I wish to express my best thanks to Dr. Schmidt for permission to make use of the statistical material. And I have also to thank cand. mag. J. Clausen for valuable assistance in the course of the work.

### Summary.

Sex chromosomes are shown to exist in the four following dioecious plants: *Humulus lupulus*, *H. Japonicus*, *Melandrium album* and *Vallisneria spiralis*.

In all four species, the male plant is heterogametic.

In the three first-named, the male plant contains a pair of chromosomes with partners of unequal size, an X- and a Y-chromosome, in addition to the autosomes.

*Vallisneria* has in the male plant an odd number of chromosomes in somatic cells. One of the chromosomes is an unpaired, X-chromosome divided by constriction into two in a peculiar manner. Not only the *Lygaeus* type, but also the *Protenor* type is thus shown to exist in the vegetable kingdom.

The sex chromosomes found are supposed to be sex determining under normal conditions, XX individuals being female, XY individuals (or XO individuals as the case may be) male.

The autosomes also, however, must, as for instance with *Drosophila* in the animal kingdom, be regarded as bearers of the tendency to male and probably female organs.

Results of countings of male and female are given, from experimental material comprising *Cannabis sativa*, *Humulus japonicus* and *H. lupulus*. All three, and especially the last, show a marked preponderance of female plants.

Copenhagen, Novr. 20 1923.

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### Postscript.

After this paper had gone to the printer I learn that Miss K. B. Blackburn in the English weekly journal »Nature« (on nov. 10. 1923) has published a provisional note (»Sex Chromosomes in Plants«) on her discovery of sex-chromosomes in one of the plant species I have mentioned in my paper, *Melandrium album*. — Miss Blackburn has investigated both the male and the female plant at the reduction division and communicates that there is no difference between the two daughter-nuclei in the female, just as one would expect; but she does not state whether the X-chromosome is the largest partner of the unequal pair, as must be supposed.

The short description and the accompanying schematic figure of the heterotypical division show that Miss Blackburn's discovery is in accordance with mine.

Ö. W.

### Explanation of Plates. (1—4).

All figures are drawn with aid of the camera lucida of Abbe, and with Zeiss' Hom. Imm. 1.5 mm. and ocular Bitumi  $\times 12.5$ . — The figures are reduced to  $\frac{9}{10}$ , except Figs. 33—35, which are reduced to ab.  $\frac{3}{5}$ .

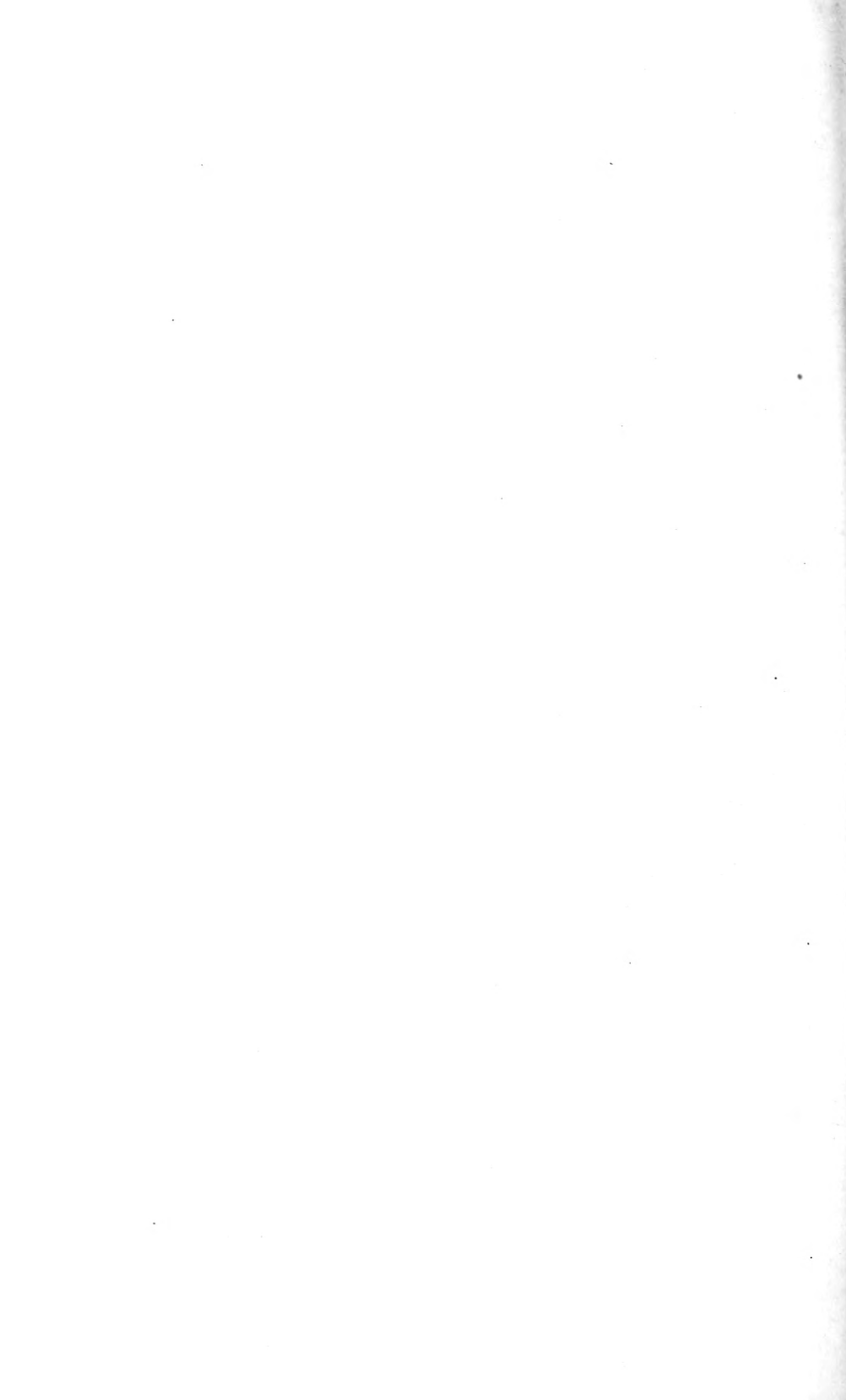
Figs. 1—5 — *Humulus lupulus* (see text pag. 5).

Figs. 6—10 — *Humulus Japonicus* (see text pag. 6-7).

Figs. 11—15 — *Melandrium album* (see text pag. 9).

Figs. 16—35 — *Vallisneria spiralis* (see text pag. 10-13).





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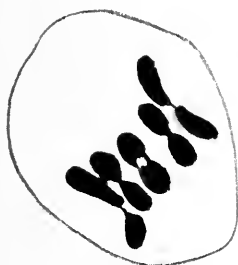
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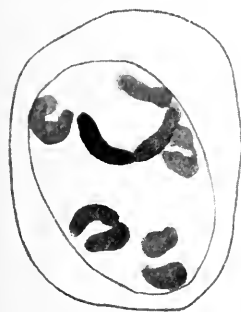
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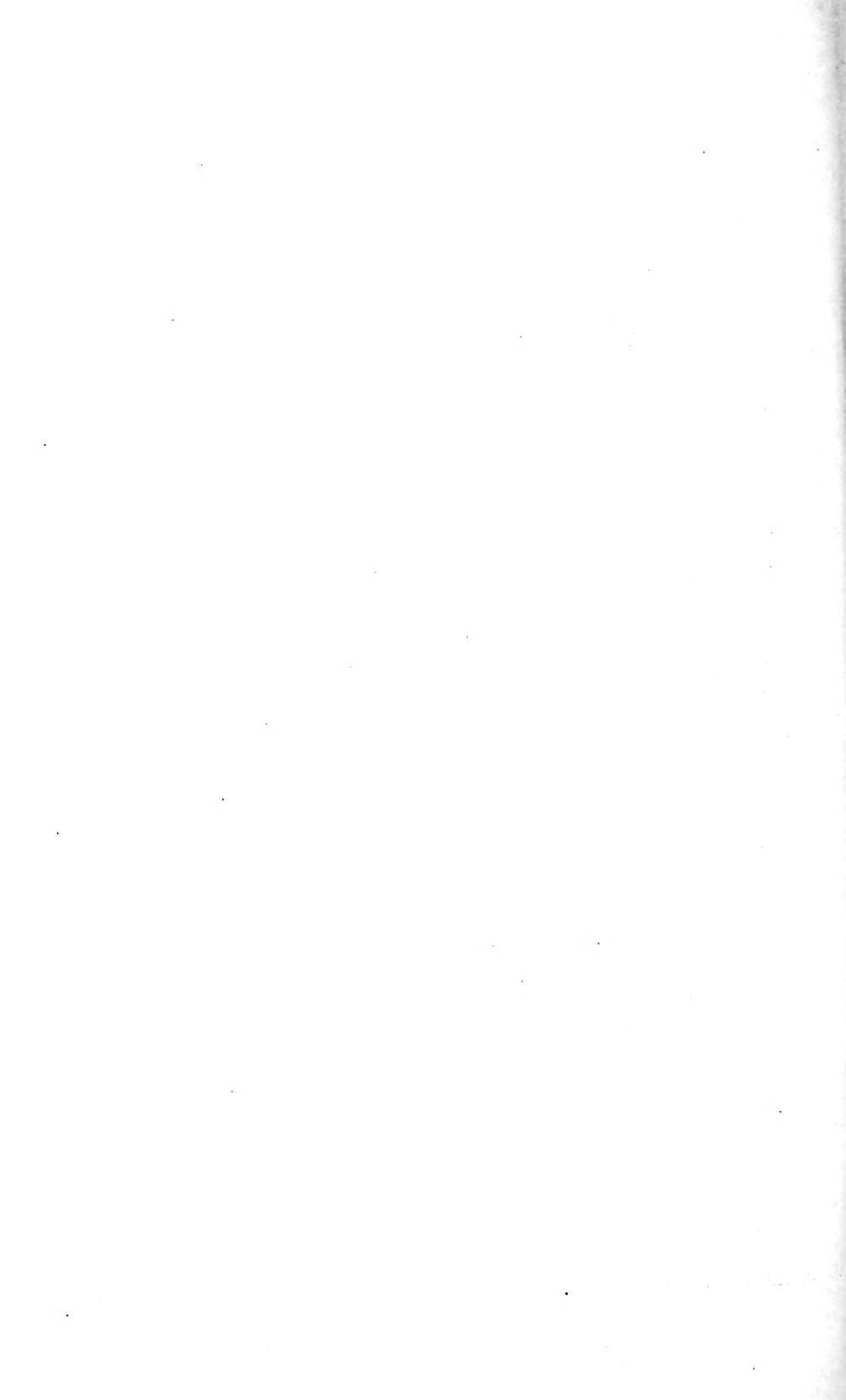


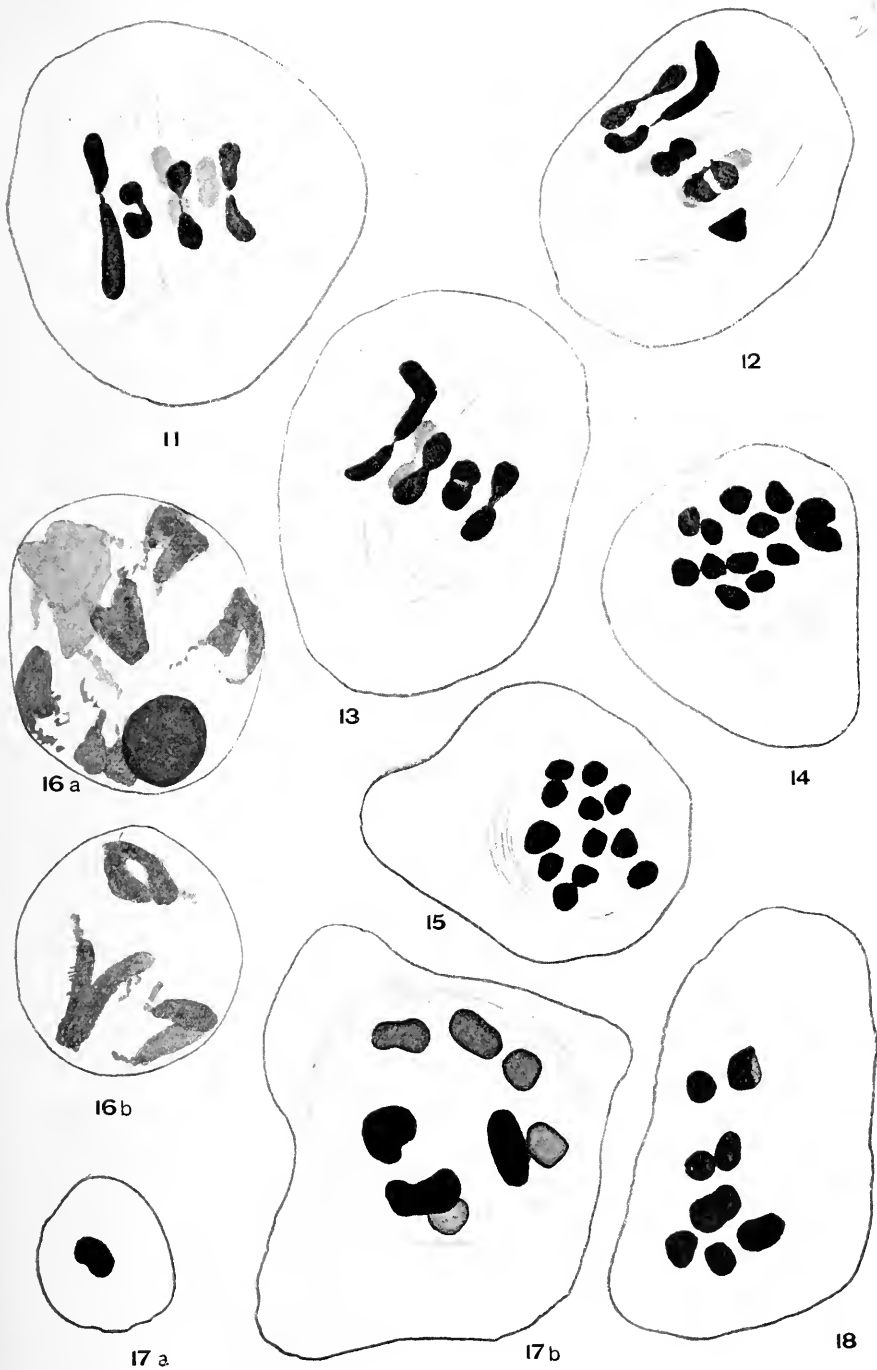
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Ö. WINGE: ON SEX CHROMOSOMES.

Ö. WINGE DEL.

MIDDELEBOE REPR.





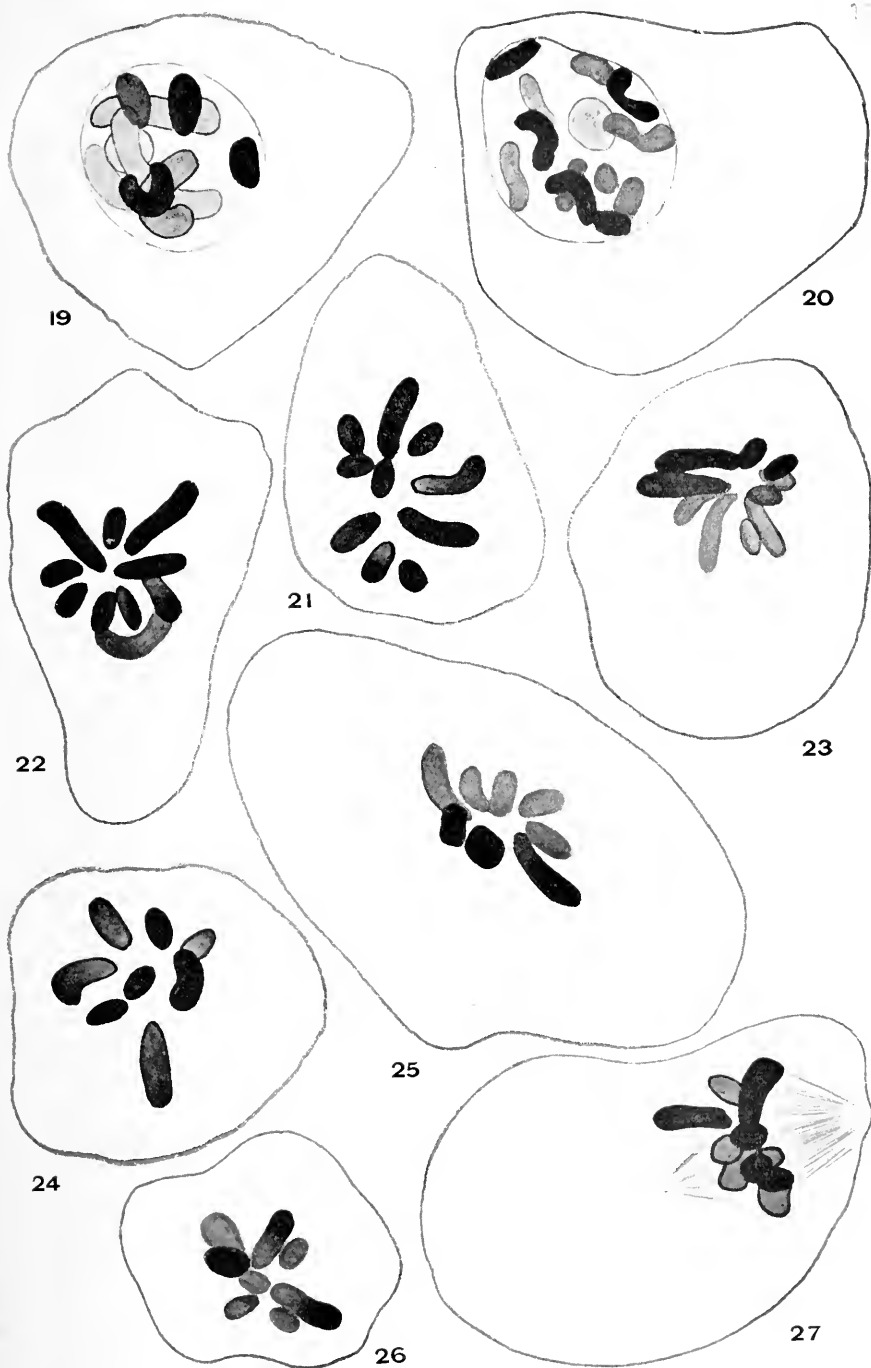
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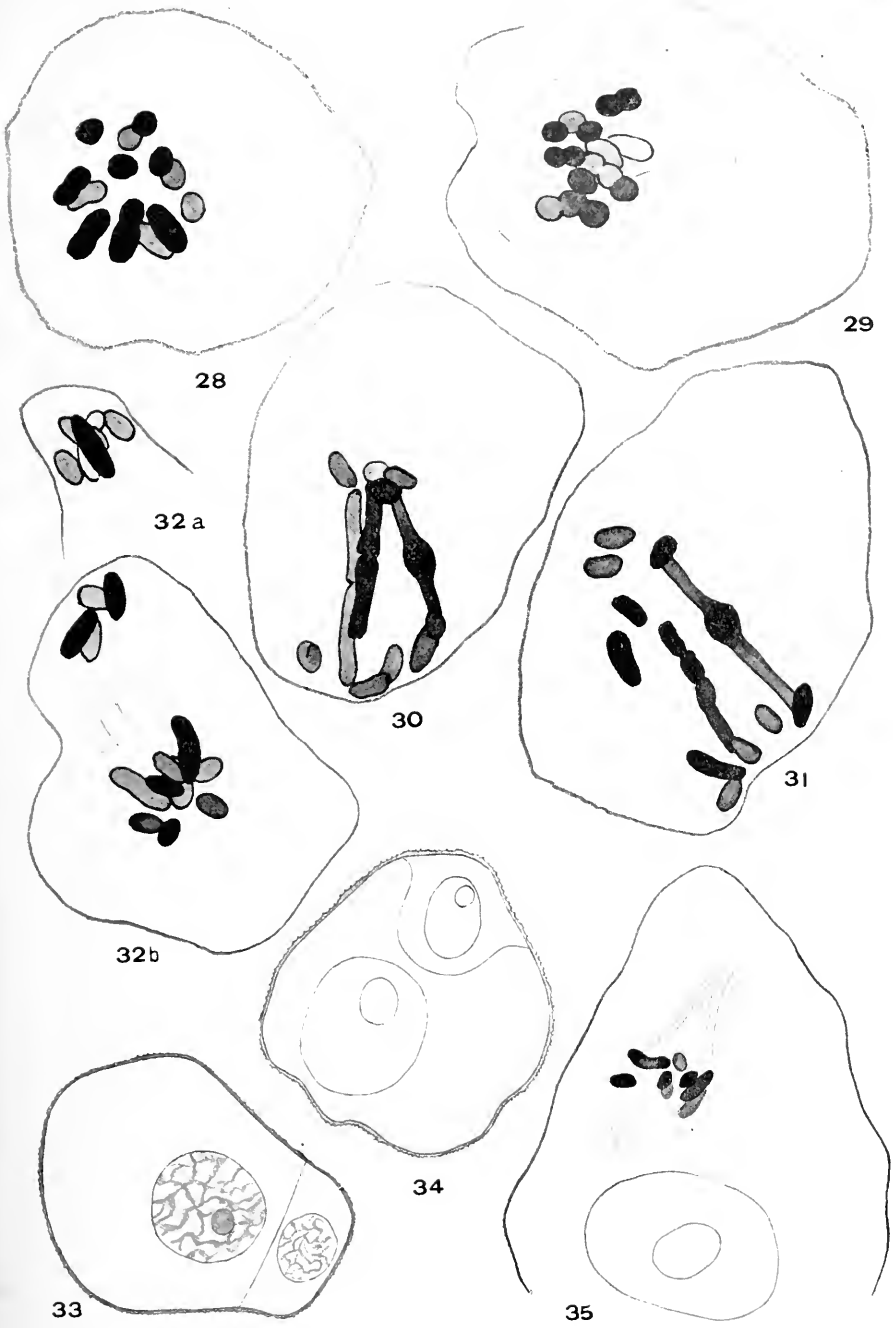


O. WINGE: ON SEX CHROMOSOMES.

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MIDDELBOE REPR.





Ö. WINGE: ON SEX CHROMOSOMES.

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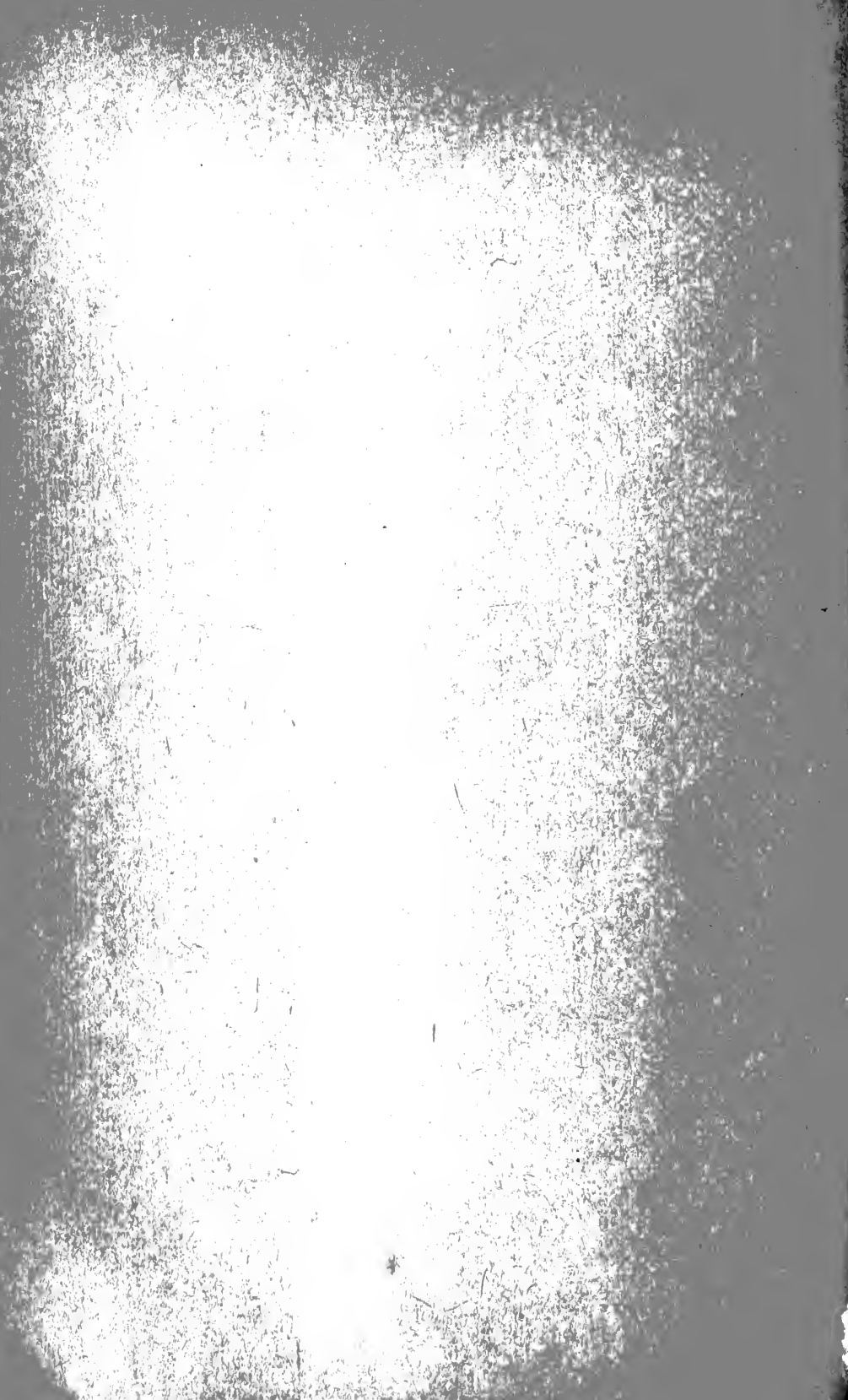
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# ON THE DETERMINATION AND VALUE OF $\pi_0$ IN ELECTROMETRIC MEASUREMENTS OF HYDROGEN ION CONCENTRATIONS.

BY

S. P. L. SØRENSEN AND K. LINDERSTRØM-LANG.

In his well-known and excellent work, »The Determination of Hydrogen Ions«, a second edition of which has recently appeared, W. Mansfield Clark sets forth in detail the difficulties at present attending the standardisation of measurements of the concentration of hydrogen ions. These difficulties are of various kinds. In the first place, there are various practical considerations in the selection of the most suitable apparatus and method; and secondly, there is the question as to the theoretical view on which the treatment of the results obtained by the measurements is based.

On the practical side, the principal questions are the following:

a) Should the 0.1 n, the 1 n, the 3.5 n or the saturated potassium chloride-calomel-mercury electrode, or possibly a different kind of electrode altogether, be used in the cell employed for measuring the concentration of hydrogen ions, and in which the hydrogen-platinum electrode constitutes the other electrode?

b) What is the most practical means of eliminating the diffusion potential?

and c) Which of the values given in the literature for  $\pi_0$  should be taken as the standard value,  $\pi_0$  being defined as the electromotive force of a cell, one half of which consists of a 0.1 n potassium chloride-calomel-mercury electrode and the other half of a hydrogen-platinum electrode in a 1 n hydrogen ion solution at a temperature of 18°, and at a hydrogen pressure of 1 atmosphere dry hydrogen, the diffusion potential being taken as eliminated.

As regards the theoretical considerations, Clark lays particular stress on the question whether it will be correct to continue to base the measurement and calculation of the concentration of hydrogen ions on the hitherto generally accepted principle that the degree of dissociation of an acid, and thus also the concentration of hydrogen ions in a solution, can be determined by measurements of conductivity, or whether the Lewis-Bjerrum activity theory should be taken as the basis for treatment of the measurement results.

After a thorough discussion of the whole question, Clark arrives at the conclusion that for the present, until the matter has been further elucidated, and to avoid confusion, it will be best to retain the method of calculation hitherto employed, and to use the standard values of  $\pi_0$  which have hitherto been used in the very great majority of determinations of concentration of hydrogen ions. Clark therefore makes the following proposal<sup>1)</sup>:

1. The following values shall be taken as the standard differences of potential, liquid junction potential differences being eliminated, between a tenth normal KCl calomel electrode and a hypothetical hydrogen electrode immersed in a solution normal with respect to the hydrogen ions, under one atmosphere partial pressure of hydrogen, and considered to have zero difference of potential between electrode and solution at all temperatures.

	Temperature					
	18°	20°	25°	30°	37.5°	40°
Potential difference . . . .	0.3380	0.3379	0.3376	0.3373	0.3364	0.3360

2. The standard experimental meaning of  $p_H$  shall be the corrected difference of potential between the hypothetical normal hydrogen electrode and the hydrogen electrode under measurement (when this difference is derived by the use of the above values), divided by the numerical quantity 0.00019837 T.

3. In every case it shall be specified whether the Bjerrum extrapolation with the use of 1.75 n and 3.5 n KCl was used to eliminate liquid junction potentials or whether saturated KCl was used and considered to eliminate liquid junction potentials.

<sup>1)</sup> l. c. p. 287.



This proposal, and the standard values for  $\pi_0$  therein given, agree in all essentials with the earliest measurements carried out at this laboratory<sup>1)</sup> and these measurements, owing to the inadequacy of the method, do not, as Clark also points out<sup>2)</sup>, show the degree of accuracy obtainable with our present methods. We have therefore thought it advisable to publish some test investigations which we have carried out during recent years. We have thus at the same time an opportunity of making some observations on the question, which is one of great importance, not least to bio-chemical research as a whole; we can also, by a positive proposal, support Clark's endeavours to arrive at a suitable standardisation in this field of work.

### A. The Calomel-mercury electrode.

W. M. Clark, in the work above mentioned, has given so detailed an account of the numerous investigations with calomel electrodes that it will hardly be necessary here to go into details regarding the extensive literature on the subject. We shall therefore, in the following, content ourselves with the briefest mention of previous results compatible with a comparison between them and those arrived at by ourselves.

With a view to showing with what degree of accuracy the calomel electrodes could be reproduced, and the durability of the same, we produced several groups of both 0.1 n and 3.5 n potassium chloride-calomel electrodes from different samples of mercury, calomel and potassium chloride.

Table 1 gives a survey of the electrodes investigated, with the materials used for their production. No further explanation of the table should be needed; it may be noted, however, that the electrolytically precipitated calomel for the e and f groups of electrodes was produced by G. F. Lipscomb and G. A. Hullett's method.<sup>3)</sup>

The potential difference of the following elements was measured:

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<sup>1)</sup> Compt. rendus du Lab. Carlsberg **8**, 1 (1909); Ergebnisse der Physiologie **12**, 393 (1912).

<sup>2)</sup> l. c. p. 281.

<sup>3)</sup> Journ. Americ. Chem. Soc. **38**, 22 (1916).

TABLE I.  
SHOWING THE CALOMEL ELECTRODES INVESTIGATED.

Group letter	Electrode letter or number	Concentration of Potassium chloride solution	Materials used		
			Mercury	Calomel	Potassium chloride
a	1, 2, 3, 4, 5, 6	0.1 n	Commercial product (rinsed in $\text{HNO}_3$ and $\text{HgNO}_3$ )	Trade product	Kahlbaum's »Zur Analyse«
b	7, 8, 9, 10	do.	do.	Own preparation (precipitated from $\text{HgNO}_3$ by NaCl)	do.
c	18, 19, 20	do.	do., twice distilled in vacuo passing through carbon dioxide (from Prof. Bjerrum, the Agricultural College)	Precipitated from $\text{HgNO}_3$ by HCl (Prof. Bjerrum)	KCl. A. R. from »The British Drug Houses« (precipitated with HCl, 5 times recrystallised and melted) (Prof. Bjerrum).
d	24, 25	do.	do.	do.	do.
e	A, B, C, D, E	do.	(Commercial product from H. Struer (twice re-distilled))	Own preparation (precipitated by electrolysis)	Kahlbaum's »Zur Analyse« (twice recrystallised)
f	F, G, H, I, K, M	do.	do.	do.	do.
g	11, 12, 13, 14	3.5 n	(Commercial product from H. Struer (once re-distilled))	Own preparation (precipitated from $\text{HgNO}_3$ by NaCl)	Kahlbaum's »Zur Analyse«
h	15, 16, 17	do.	do.	do.	do. once recrystallised
i	21, 22, 23	do.	do., twice distilled in vacuo passing through carbon dioxide (Prof. Bjerrum)	Precipitated from $\text{HgNO}_3$ by HCl (Prof. Bjerrum)	KCl as Group c
k	26	do.	do.	do.	do.

Hg/ HgCl, 0.1 n KCl/	0.1 n KCl/	0.1 n KCl HgCl/ Hg	(I)
18°	18°	18°	
Hg/ HgCl, 3.5 n KCl/	3.5 n KCl/	3.5 n KCl HgCl/ Hg	(II)
18°	18°	18°	
Hg/ HgCl, 0.1 n KCl/	0.1 n KCl/	0.1 n KCl HgCl/ Hg	(III)
25° eller 30°	18°	18°	
Hg/ HgCl, 3.5 n KCl/	3.5 n KCl/	3.5 n KCl HgCl/ Hg	(IV)
25° eller 30°	18°	18°	
Hg/ HgCl, 0.1 n KCl/	3.5 n KCl/	3.5 n KCl HgCl/ Hg	(V)
18°	18°	18°	

In all these measurements, which were carried out at 18°, both cells, which were of the form generally used in this Laboratory (Ostwald's model, see Clark p. 194, Model G) were placed in the same well regulated water thermostat, the connecting liquid being in a vessel outside. In the measurements for determining the temperature coefficient of the electrodes, the cells were placed in separate thermostats, and the connecting liquid in a vessel between the two. The measurements of potential difference were carried out in the usual manner by means of an Otto Wolff's potentiometer with a mirror galvanometer as the zero instrument and a Weston cell as the normal element.

*a. Constancy and durability of the calomel electrodes.*

During the last few years, we have made a great number of measurements with cells of Types I and II to show the constancy and durability of the different 0.1 n (or 3.5 n) potassium chloride-calomel electrodes. The method here employed was to measure one of the given electrodes against each of the others of its type, and this was repeated from time to time.

It will not be necessary here to give the great mass of numerical material obtained; it will suffice to note that none of the electrodes produced ever showed a difference of potential of 1 millivolt as compared with the electrode selected, and, save in Group a, which was prepared from commercial products subjected to no further purification, the potential difference as measured never exceeds  $1/2$  millivolt, and is generally far below this.

We did not succeed, however, in obtaining the extraordinarily high degree of uniformity between the different electrodes arrived at by N. E. Loomis and S. F. Acres<sup>1)</sup> or later, by

<sup>1)</sup> Americ. Chem. Journ. **46**, 594 (1911).

C. N. Myers and S. F. Acres<sup>1)</sup> these investigators reckoning with a maximal deviation of 0.1 millivolt, while in the great majority of cases they found the difference between individual electrodes even less. In researches of quite special character, it is undoubtedly of the highest importance to have apparatus of such a degree of precision as that used by Acres and his collaborators, and in such cases, it is worth while to devote the time and trouble demanded for the preparation and use of Acres' instruments. With ordinary investigations, however, this will hardly be the case, as sources of error of a different character, and on other points, render the advantage of a high degree of accuracy on a single point illusory.

Our own researches warrant, in our opinion, the conclusion that one can, by the use of fairly purified materials, easily and with little trouble produce calomel electrodes with a potential difference, which, even after they have been kept for months, will rarely exceed  $\frac{1}{2}$  millivolt, and this, moreover, both for 0.1 n and 3.5 n calomel electrodes. This agrees in all essentials with the results of some experiments carried out almost at the same time as ours, at the Chemical Laboratory of Copenhagen University<sup>2)</sup> and the Chemical Laboratory of the Danish Agricultural College<sup>3)</sup>, the agreement however, being somewhat better in the last-named researches, in which the materials used were subjected to a more searching process of purification than used in our own.

*b. Temperature coefficient of the calomel electrodes.*

For determination of the temperature coefficient, the cells (III) and (IV) (see p. 5) were repeatedly measured, using most of the electrodes produced, which showed on the whole the same temperature coefficient independent of the materials used for preparation of the electrodes. As regards the method itself, it need only be said that the electrodes used for measurement purposes were first compared at 18°, and the difference of potential between two electrodes found on this comparison introduced as a correction in the subsequent measurement of potential difference between these two electrodes at different temperatures. The temperatures in the two thermostats were kept constant to a degree of accu-

<sup>1)</sup> Ibid. **50**, 403 (1913).

<sup>2)</sup> Stig Veibel: Journ. Chem. Soc. London **123**, 2203 (1923).

<sup>3)</sup> J. K. Gjaldsbæk: Vidensk. Selskab, Mathem.-fysiske Meddelelser **5**, Nr. 9 (1924).

racy of  $0.05^{\circ}$ , and the electrodes, when moved from one thermostat to the other, very soon — generally in 10–15 minutes — assumed a constant potential. The results of the measurements were as follows:

Potential difference in volts between 0.1 n calomel electrodes at different temperatures:

E	E
$25^{\circ}$	$18^{\circ}$
0.00544	
554	
557	
551	
562	
560	
559	

Mean 0.00555, Temperature coeff: 0.00079.

E	E
$30^{\circ}$	$18^{\circ}$
0.00943	
967	
960	

Mean 0.00957, Temperature coeff: 0.00080.

Potential difference in volts between 3.5 n calomel electrodes at different temperatures:

E	E
$25^{\circ}$	$18^{\circ}$
0.00321	
340	
327	
332	
326	
301	
317	
302	

Mean 0.00321, Temperature coeff: 0.00046.

E	E
$30^{\circ}$	$18^{\circ}$
0.00577	

Temperature coeff: 0.00048.

The temperature coefficients here found agree well with the figures given by previous investigators. Th. W. Richards, for instance,<sup>1)</sup> finds, for the 0.1 n electrode, a temperature coefficient of 0.00079, and J. K. Gjaldbæk<sup>2)</sup> finds, for the 0.1 n electrode 0.00077, and for the 3.5 electrode 0.00045.

The temperature coefficient thus, as already shown by Th. W. Richards in the above-mentioned work, increases with the dilution of the electrolyte, and as far as that goes, the 1.0 n or the 3.5 n electrode would be preferable to the 0.1 electrode. Actually, however, as will be shown more precisely in the following, the position is somewhat different when the calomel electrode enters, as half-electrode, into a calomel-hydrogen cell, and the temperature coefficient of the whole cell is in question.

*c. Potential difference between the 0.1 n and the 3.5 n potassium chloride-calomel electrode.*

For determination of this potential difference at 18°, the cell V (see p. 5) was measured. The results of the measurements are shown in Table 2, the columns of figures here requiring no further explanation. It will be seen that the mean value of all measurements is 82.98 millivolts, that is to say, the 0.1 n electrode has a potential 0.0830 volts greater than that of the 3.5 n electrode, this, however, including the diffusion potential between a 0.1 n and a 3.5 n potassium chloride solution.

The mean value arrived at, 0.0830 volts, agrees well with the value first found by N. Bjerrum and J. K. Gjaldbæk<sup>3)</sup> of 0.0830. Some measurements carried out at the University Chemical Laboratory some years ago, however, gave 0.0845 volts, and some test measurements in connection therewith, made at the chemical laboratory of the Agricultural College, gave 0.0841 volts.<sup>4)</sup> These values are nevertheless probably too high, as S. Veibel<sup>5)</sup> working at the University Chemical Laboratory, has since found a considerably lower potential difference, (the mean value for the 24 hours electrode was 0.0833) and J. K. Gjaldbæk<sup>6)</sup> has, in his

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<sup>1)</sup> Zeitschr. physik. Chem. **24**, 49 (1897).

<sup>2)</sup> l. c. (1924).

<sup>3)</sup> Den kongl. Veterinær- og Landbohøjskoles Aarskrift 1919, p. 73.

<sup>4)</sup> E. Billmann: Københavns Universitets Festskrift 1920, p. 80—81.

<sup>5)</sup> Jour. Chem. Soc. **123**, 2207 (1923).

<sup>6)</sup> l. c. (1924).

TABLE 2.

The potential difference between 0.1 n - and 3.5 n - potassium chloride-calomel-electrodes, without taking account of the diffusion potential.

Numbers of the 0.1 n Calomel electrodes used	Numbers of the 3.5 n Calomel electrodes used	Mean of the potential difference found by the measurement
7-8-9-10	12-13-14-15-16-17	83.12
do.	11-12-13-14-15-16-17	82.94
do.	do.	83.07
do.	do.	82.90
do.	do.	83.04
do.	do.	83.13
18-19-20	21-22-23	82.91
7-8-9-10	15-16-17	83.22
18-19-20	21-22-23	82.93
7-8-9-10	13-14-16-17	83.11
18-19-20	21-22-23	82.95
7-8-9-10-18-19-20	13-15-16-17	82.98
do.	11-12-14-21-22-23	82.70
do.	do.	82.73
do.	13-15-16-17-26	83.02
Mean...		82.98

latest, careful and comprehensive measurements at the Agricultural College obtained the mean value 0.08313. We may therefore, we think, regard the 0.0831 volts as very nearly correct.

We have not ourselves determined the potential difference between the 0.1 n and the 1.0 n potassium chloride-calomel electrode, but good determinations of this are to be found in extant literature. Gilbert N. Lewis and Merle Randall<sup>1)</sup> for instance, give the potential difference at 25° as 0.0530 volts, while Gilbert N. Lewis, Thomas B. Brighton and Reuben L. Sebastian<sup>2)</sup> give 0.0529 for the same temperature. If we now take the temperature coefficients found by Th. W. Richards<sup>3)</sup> for the 0.1 n electrode (0.00079) and for the 1.0 n electrode (0.00061) we find as the potential difference between the

<sup>1)</sup> Journ. Americ. Chem. Soc. **36**, 1974 (1914).

<sup>2)</sup> Ibid. **39**, 2255 (1917).

<sup>3)</sup> Zeitschr. physik. Chem. **24**, 49 (1897).

0.1 n and the 1.0 n potassium chloride-calomel electrode at 18°:  
 $0.0529 \div 7 (0.00079 \div 0.00061) = 0.0529 \div 0.0013 = 0.0516$  volts.

The saturated potassium chloride-calomel electrode introduced by L. Michaelis and W. Davidoff<sup>1)</sup> has not been included in our investigations, as this electrode, which offers certain advantages as a working electrode, is hardly suitable, even in the opinion of Michaelis himself,<sup>2)</sup> as a standard.

It must further be noted that Stig Veibel<sup>3)</sup> has quite recently suggested using the Biilmann quinhydrone-electrode, which is extremely easy to prepare, and very soon attains a constant potential, for purposes of comparison. And it is certainly beyond doubt that on many occasions, especially with measurements of hydrogen ions by means of the quinhydrone electrode, it may be advisable to substitute, in place of the calomel electrode, a quinhydrone electrode with known electrode liquid (e. g. as suggested by Veibel, 0.01 n hydrochloric acid — 0.09 n potassium chloride solution) but as the quinhydrone electrode cannot be trusted to remain constant more than 24 hours at the outside, and as the accuracy with which this electrode can be reproduced is no greater than that in the case of the calomel electrode, which lasts for months, it would seem more reasonable to use a calomel electrode as the standard.

*d. Which calomel electrode should then be used?*

The question then is, which of the calomel electrodes should be given the preference. Different writers are by no means agreed as to which of the calomel electrodes is the most constant, and best retains its potential when exposed to shaking, or quickest to resume its original potential after exposure to alterations of temperature. In our comparisons between the 0.1 n and the 3.5 n calomel electrode, we found no reason for preferring either as long as the electrodes were treated with the necessary care. The risk of an alteration in the electrode potential by diffusion of potassium chloride over into the electrode liquid from the strong potassium chloride solution is of course greater with the 0.1 n than with the 1 n or the 3.5 n electrode, and as far as this point is concerned, an electrode with

<sup>1)</sup> Biochem. Zeitschr. **46**, 148 (1912).

<sup>2)</sup> See L. Michaelis & R. Krüger. Ibid. **119**, 310 (1921).

<sup>3)</sup> Journ. Chem. Soc. **123**, 2203 (1923).



a high concentration of potassium chloride is to be preferred. But when it is also pointed out, in the case of these last electrodes, that their temperature coefficient is lower than that of the 0.1 n electrode, it should be borne in mind that this advantage may be of doubtful value, since, in using the electrode, the temperature coefficient of the other half-cell has also to be taken into consideration. Thus for instance, the hydrogen electrode, with an electrode liquid 1 n with regard to hydrogen ions, has a temperature coefficient of almost the same magnitude as the 0.1 calomel electrode, so that the temperature coefficient for the whole standard cell consisting of a 0.1 n calomel electrode as the one half-cell and the hydrogen electrode in a 1.0 hydrogen ion solution as the other, is only very slight, whereas the temperature coefficients for the corresponding cells with the 1.0 n — or the 3.5 n — calomel electrode are considerably higher.<sup>1)</sup>

Taking the values given in the introduction<sup>2)</sup> for potential difference at 18°, 20° and 30°, between a 0.1 n calomel electrode and a hydrogen electrode in a 1.0 n hydrogen ion solution, we find that this element has a temperature coefficient of — 0.00006, and as the 0.1 n calomel electrode has a temperature coefficient of + 0.00079, the temperature coefficient of the hydrogen electrode will be — 0.00085. The corresponding cells with a 1.0 n or a 3.5 n calomel electrode respectively will then have the following temperature coefficients:

$$\begin{aligned} (1\text{ n}): + 0.00061^{3)} - 0.00085 &= - 0.00024 \\ \text{and } (3.5\text{ n}): + 0.00046^{4)} - 0.00085 &= - 0.00039 \end{aligned}$$

With our present knowledge of the different calomel electrodes, there seems then, in our opinion, no very weighty ground for preferring one electrode to the other. As, moreover, it is very easy, from the data available, to compare the values found

<sup>1)</sup> Owing to the facts here noted, it seems to us hardly practical, in the definition for  $\pi_0$ , to introduce, as Clark has done (l. c. p. 287) the supposition that the potential between hydrogen platinum electrode and the 1 n hydrogen ion solution should be take as nil at all temperatures, since the whole temperature coefficient of the cell would thus fall upon the calomel electrode, the true temperature coefficient of which is, as above mentioned, quite different from that of the cell.

<sup>2)</sup> See p. 2.

<sup>3)</sup> From the measurement by Th. W. Richards: *Zeitschr. physisk Chem.* **24**, 49 (1897).

<sup>4)</sup> J. K. Gjaldbæk (l. c.) finds 0.00045; we found, as the mean, 0.00047 (see p. 7).

from the different calomel electrodes, we consider it unnecessary to make any suggestion as to confining future operations to one or the other of the calomel electrodes here referred to.

When, by measuring the chain  $\text{Hg}, \text{HgCl } 1.0 \text{ n}$  (or  $3.5 \text{ n}$ )  $\text{KCl} / x \text{ n KCl}$  arbitrary half-cell at the temperature  $t^\circ$ , the potential difference  $\pi$  has been arrived at, it will then be possible, by measurement of the same cell, only using a  $0.1 \text{ n}$  calomel electrode in place of the  $1.0 \text{ n}$  or  $3.5 \text{ n}$ , at the same temperature, to find the potential difference:

$$(0.1 \text{ n in place of } 1.0 \text{ n}): \pi + 0.0516 + (t - 18) (0.00079 - 0.00061)$$

$$(0.1 \text{ n in place of } 3.5 \text{ n}): \pi + 0.0831 + (t - 18) (0.00079 - 0.00046)$$

These formulæ, which can at any rate be used between  $15^\circ$  and  $30^\circ$ , give the additional term  $a$  by means of which it is possible to calculate the potential difference of the cell when using the  $0.1 \text{ n}$  calomel electrode, when the potential difference for one of the other calomel electrodes is known. Table 3 gives the values of this additional term compared for some temperatures between  $15^\circ$  and  $30^\circ$ .

TABLE 3.

THE ADDITIONAL TERM  $a$ .

If  $\pi$  be the potential difference found, when using a  $1.0 \text{ n}$ - or a  $3.5 \text{ n}$ - potassium chloride-calomel-electrode, then the potential difference with a  $0.1 \text{ n}$ - potassium chloride-calomel-electrode will

be  $\pi + a$

Temperature $t$	The additional term $a$	
	For the $1.0 \text{ n}$ - calomel-electrode	For the $3.5 \text{ n}$ - calomel-electrode
15	0.0511	0.0821
16	512	824
17	514	828
18	516	831
19	518	834
20	520	838
21	521	841
22	523	844
23	525	848
24	527	851
25	529	854
26	530	857
27	532	861
28	534	864
29	536	867
30	538	871

By means of the additional terms given in Table 3, it is easy to compare values found when using the different sorts of calomel electrodes, and we therefore propose, as already mentioned, that no selection should be made from among them; in all measurements, however, it should always be stated which calomel electrode has been used.

In the great majority of biochemical measurements it will be sufficient to have only one calomel electrode, this being compared, at monthly intervals, with a freshly prepared calomel electrode, and if necessary replaced by the latter.

In more precise measurements, it is best not to be content with a single calomel electrode, but to have a collection of at least four, preferably more, so that frequent comparison can be made between them, and any exhibiting a deviation in potential difference from the rest be discovered and rejected. The whole collection must, of course, be compared at suitable intervals with freshly prepared electrodes.

The most rational mode of proceeding, however, with precise measurements, will be to use the calomel electrode only as a working electrode, an auxiliary, whose potential difference is not reckoned as known, but is daily determined in relation to a hydrogen electrode, this latter thus being the true standard electrode. As will be further set forth in Section C, it is proposed, for this purpose, to use the hydrogen electrode in a solution 0.01 *n* for hydrochloric acid and 0.09 *n* for potassium chloride.

## B. The Diffusion Potential.

In potential measurements in electrolyte solutions, especially solutions with high content of hydrogen or hydroxyl ions, the determination or elimination of the diffusion potential between solutions of different electrolyte concentration will of course often render the measurement more or less uncertain.

None of the formulæ hitherto proposed for calculation of the diffusion potential is applicable beyond a limited degree, and furthermore, it is difficult in practice to establish such ideal contact surfaces as have been reckoned with in the formulæ themselves.

The method proposed by J. N. Brønsted<sup>1)</sup> some years back for calculating the diffusion potential can likewise hardly be con-

<sup>1)</sup> Journ. Americ. Chem. Soc. **44**, 897 (1922).

cidered as generally applicable. Theoretically, the method he suggests is extremely interesting, but it is too circumstantial for use in everyday practice, being based on determinations of solubility of sparingly soluble substances in such electrolyte solutions as those between which the diffusion potential is to be found.

In ordinary laboratory work therefore, it will generally be necessary to fall back on a method in which the diffusion potential is as far as possible eliminated. As however, in our present state of knowledge, it is impossible to say definitely whether such elimination is complete or not, it is necessary to choose a method which shall be applicable with the widest possible scope, and can, under varying conditions, be taken as giving the highest possible degree of elimination, at the same time yielding constant and reproducible results without too much trouble or too much expenditure of the liquid under investigation.

There is no need for us here to give any historical account of the various methods which have been proposed from time to time; it must suffice to note, that the usual procedure is either to use a saturated solution of potassium chloride as connecting liquid, and regard the diffusion potential as eliminated thereby, or, as first proposed by N. Bjerrum<sup>1)</sup> to use both a 1.75 n and a 3.5 n potassium chloride solution as connecting liquid, and then take the difference between the two measurements as an extrapolation term to render the elimination more complete than could be arrived at by using a saturated solution of potassium chloride alone. In many cases, and especially with potential measurements in practically all solutions used for biological investigations, the two methods give the same results, which suggests that the elimination has really been successful, but in liquids of markedly acid or markedly alkaline character this is not so. Here, as was to be expected, a more complete elimination is obtained by the use of the Bjerrum extrapolation method than by using the saturated potassium chloride solution alone. We have always preferred to use this method, as being more generally applicable, and also because, as further noted in the following, the determination of  $\pi_0$  necessitates the use of the extrapolation method.

In this connection, it must further be noted that a somewhat different method was proposed a year ago by L. Michaelis<sup>2)</sup>,

<sup>1)</sup> Zeitschr. physik. Chemie. **53**, 428 (1905).

<sup>2)</sup> L. Michaelis and Kakinuma: Biochem. Zeitschr. **141**, 394 (1923); L. Michaelis and A. Fujita: *ibid.* **142**, 398 (1923).

this demanding only a single measurement and no extrapolation. Michaelis uses a saturated potassium chloride solution as connecting liquid, but places between it and the electrode liquid a solution of the electrode liquid saturated with potassium chloride. Michaelis sets forth the reasons suggesting that this alteration in the arrangement of the experiment should greatly reduce the diffusion potential, and gives a comparatively simple and practical mode of carrying out the measurement. A series of experiments by L. Michaelis and A. Fujita<sup>1)</sup> show on the whole that this process agrees well with the Bjerrum extrapolation method; some few experiments we have made ourselves point in the same direction. The saving of time obtained by Michaelis' new method however, is very slight, and we have — as already mentioned — preferred, at any rate for the present, to use the thoroughly tested extrapolation method.

In the elimination of the diffusion potential, it is of essential importance that the contact surface between the two solutions should be established in a certain manner, and the subsequent measurement made after a certain interval of time. In this connection, interesting information is afforded by the experiments of A. M. Chuno<sup>2)</sup> with symmetrical chains.



Such a chain should of course not show any difference of potential but the writer easily succeeded in obtaining a very distinct potential difference, when the sharpest possible boundary surface was maintained at the one point of contact (A), this not being the case with the other (B). Several other writers confirm this result.

Many different methods have been suggested for the establishment of this boundary surface (threads moistened with one of the solutions; U-tubes filled with sand, agar or similar substances saturated with one solution; collodium films and similar membranes as partition walls between the two solutions; and others besides), but the usual course, it would seem, is to establish direct surface contact between the two liquids, this method involving fewest complications, and affording the easiest means of

<sup>1)</sup> l. c.

<sup>2)</sup> Ann. Univ. Lyon, n. s. 1, 18 (1906); quoted from W. M. Clark: The Determination of Hydrogen Ions 1922, p. 167.

renewing the boundary surface in question. An extremely fine modification of this procedure has been suggested by A. B. Lamb and A. T. Larson<sup>1)</sup> who, in a comparatively simple manner, by slow and steady confluence of the two solutions, produce a constant renewal of the contact surface, a „flowing junction”, thus permitting as constant and reproducible a state of the contact surface as possible. We have thoroughly tested this method with the greatest care, and can give it our best recommendation, as yielding particularly constant values for the diffusion potential, as long as the rate of flow is kept fairly constant itself<sup>2)</sup>. There is, however, this disadvantage with the method, that it demands a considerable expenditure not only of the connecting liquid, but also of the electrode liquid, the supply of which is often very limited.

In our opinion therefore, save for measurements of a quite special character, it is advisable to use the customary method, contact between the electrode liquid and the intermediary being established by simply dipping the siphon tube of the electrode vessel, filled with the electrode liquid, into or slightly under the surface of the somewhat heavier connecting liquid, and the measurement taken immediately (i. e. one or two minutes after contact is established). If the measurement is to be repeated after the lapse of some little time, the siphon tube of the electrode vessel should be lifted from the connecting liquid, and before replacing it in the same, some drops of the electrode liquid should be allowed to run off.

We should further point out that we have obtained the most constant results when the point of the siphon tube was not too narrow, and it has also been found advisable to arrange the experimental apparatus in such a manner that the cock of the electrode vessel could be left open during the short time occupied by the actual measurement.

In the experimental results quoted below, and in the following section, the terms  $\pi_{1.75}$  and  $\pi_{3.5}$  are used to denote the electromotive force of the element measured, with 1.75 n and 3.5 n potassium chloride solution respectively as connecting liquid, the difference  $\pi_{1.75} - \pi_{3.5}$  constituting the above mentioned extrapo-

<sup>1)</sup> Journ. Americ. Chem. Soc. **42**, 229 (1920).

<sup>2)</sup> cf. Duncan A. Mac Innes and Yu Liang Yeh: Journ. Americ. Chem. Soc. **43**, 2563 (1921).

lation term, being denoted by  $\Delta\pi$ . The extrapolated value  $\pi_{\text{extrap}} = \pi_{3.5} - \Delta\pi$  is regarded as the true electromotive force of the element, corrected for any diffusion potential which might be present. The correctness of this assumption is not proved, and can hardly be proved at the present state of our knowledge, so that we have here really a question of definition. On the other hand, there can be little doubt but that the elimination of the diffusion potential may be regarded as complete or very nearly so, as long as  $\Delta\pi$  is nil or of low value, though we can hardly be sure of this when  $\Delta\pi$  attains a magnitude of several millivolts. In standardisation determinations, this should be taken into consideration, and the electrode liquid so chosen as to render the value of  $\Delta\pi$  as low as possible, in any case not exceeding 1 millivolt.

In order to avoid superfluous figures, we have in the following survey of our measurement results (Tables 4—7, p. 28—31) noted only the values for  $\Delta\pi$  and for  $\pi_{\text{extrap}}$ ; the actual measured values can be easily calculated from this, as

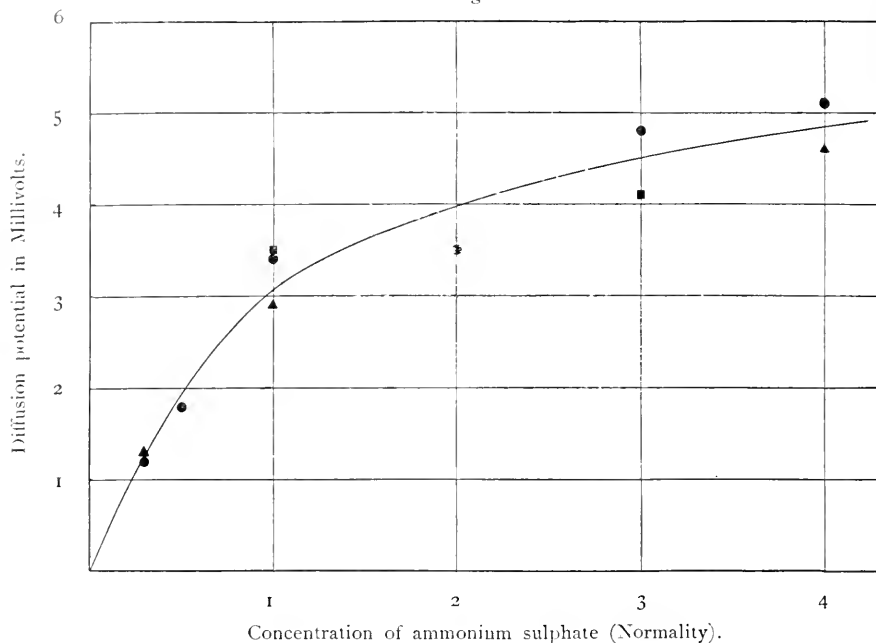
$$\begin{aligned}\pi_{3.5} &= \pi_{\text{extrap}} + \Delta\pi \\ \pi_{1.75} &= \pi_{\text{extrap}} + 2 \Delta\pi\end{aligned}$$

The third and fourth vertical columns of Table 4 (p. 28) show how the magnitude of  $\Delta\pi$  varies with the composition of the electrode liquid. The values given for  $\Delta\pi$  are mean values of all the experiments made. As regards the agreement between the values for  $\Delta\pi$  in the separate experiments, we may mention that in the case of the best investigated experimental mixture (0.01 n HCl + 0.09 n KCl) the result was as follows: 6 experiments made in the years 1921, 1922 and 1924 with flowing junction gave values for  $\Delta\pi$  only varying between 0.00060 and 0.00066 volts, while 36 experiments carried out during the same period with ordinary dipping gave values for  $\Delta\pi$  varying between 0.00040 and 0.00090 volts, the mean value being 0.00066 volts. The accuracy obtained by using the flowing junction is thus far greater than with the ordinary dipping method, but the mean value of  $\Delta\pi$  is in both cases very nearly the same. We have therefore not hesitated to use the flowing junction method in a great number of the measurements on which the calculation of  $\pi_0$  (see following section) is based, though the value for  $\pi_0$  as found was intended to be used, and is still used at this laboratory for measurements

of hydrogen ions in solutions of which the quantity available is so small as to preclude the use of the flowing junction method.

What has here been said as to the agreement between the separate experiments in the investigation of the electrode liquid (0.01 n HCl + 0.09 n KCl) also applies on the whole to the other

Fig. 1.



experiments noted in the table; we must, however, point out that the agreement becomes less satisfactory with increasing acidity of the electrode liquid. Thus for instance, with the 8 experiments noted in the table for the electrode liquid (0.1 n HCl) 5 experiments with the flowing junction method gave values for  $\Delta\pi$  varying between 0.00402 and 0.00440 volts, whereas 3 experiments with ordinary dipping gave values for  $\Delta\pi$  varying between 0.00445 and 0.00482 volts.

On looking through the tables 5, 6 and 7 (p. 29—31) it will be noticed — as indeed was to be expected — that  $\Delta\pi$  shows a marked decrease when the concentration of potassium chloride in the electrode solution is increased relatively to the concentration of hydrochloric acid, and with a sufficiently high concentration of the potassium chloride it becomes 0, whereas an in-



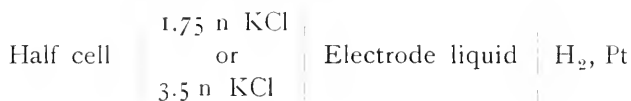
crease in the sodium chloride concentration of the electrode liquid leads to negative values for  $\Delta\pi$ ; this may be explained by the fact that the mobility of the sodium ion is far less than that of the chlorine ion, whereas the potassium and chlorine ions have very nearly the same velocity.

Precisely analogous conditions are observed in the case of electrode solutions containing ammonium sulphate, the sulphate ion in solutions of any strength having a much lower mobility than that of the ammonium ion, so that  $\Delta\pi$  in such cases becomes positive, and can, at high concentrations of ammonium sulphate, attain a considerable magnitude, even though the concentration of hydrogen ions be so slight that the hydrogen ions are of no importance in this connection. To further elucidate this point, we have set out graphically, in Fig. 1, some results of measurements with 0.1 n prim. sodium citrate containing various quantities of ammonium sulphate as electrode liquid. We have here taken the normality of ammonium sulphate as abscissa, and the value of  $\Delta\pi$  in millivolts (concentration of hydrogen ions, answering to  $p_H = 4.6-4.9$ ) as ordinate. The circles denote experiments with ordinary dipping, and a comparatively narrow siphon tube, the squares experiments with ordinary dipping and a wide tube, the triangles indicating experiments with flowing junction.

### C. Magnitude of $\pi_0$ .

#### a. Mode of calculation.

Using  $\pi$  to denote the extrapolated electromotive force (see Section B) of an element of the type



the concentration of hydrogen ions in the electrode liquid  $C_H$  can then, as we know, be calculated by means of the formula:

$$\log \frac{1}{C_H} = p_H = \frac{\pi - \pi_0}{K} \quad (I)$$

where  $K = 0.0577 + 0.0002 (t^\circ - 18^\circ)$  and  $\pi_0$ , which varies to some extent with the temperature, denotes the extrapolated electromotive force of a hypothetical cell constructed exactly as the above, and with an electrode liquid having a concentration of hydrogen ions equal to 1 n.

Determination of the value of  $\pi_0$  is likewise effected by measuring the extrapolated electromotive force of cells of the above noted type, using electrode liquids of known or calculable concentration of hydrogen ions. If the extrapolated electromotive force of such a cell be  $\pi$ , and the known concentration of hydrogen ions in the electrode liquid  $C_H$ , then  $\pi_0$  can be calculated from the following formula:

$$\pi_0 = \pi - K \log \frac{1}{C_H} \quad (II).$$

The difficulties attaching to this apparently simple method of determining  $\pi_0$  lie not only in the feature noted in the foregoing section as to selection of a constant and durable half cell on the left and complete elimination of the diffusion potential, but also in the selection of a suitable electrode liquid for the right half cell, with a concentration of hydrogen ions which can be calculated with sufficient accuracy. In some respects, adequate buffer mixtures — phosphate, acetate, phthalate mixtures etc. — present great advantages, as their concentration of hydrogen ions is only slightly altered by small quantities of impurities from the air, or from the glass vessels employed, while the diffusion potential also, with such mixtures, is as a rule insignificant, or can at least be completely eliminated without difficulty. Such mixtures are therefore admirably adapted as electrode liquids for the purpose of test experiments now and again in everyday practice, but can hardly be used for fundamental determinations of  $\pi_0$ , as the calculation of concentration of hydrogen ions in such a mixture demands a knowledge of the dissociation constant of the acid concerned under the given circumstances; and since our knowledge of this is as a rule based precisely on electrometric measurements of hydrogen ions, the constants thus found can of course never be used for determination of  $\pi_0$ .

In the fundamental measurements for determination of  $\pi_0$  there is therefore hardly any other way open to us than the use of electrode liquids whose concentration of hydrogen ions can be ascertained by other means, and which are otherwise suited to the purpose. The most suitable and most generally used electrode liquid is undoubtedly a highly diluted hydrochloric acid solution, containing suitable quantities of potassium- or sodium chloride. The concentration of hydrochloric acid must, for the sake of the diffusion potential, be slight, but on the other hand

not so slight as to render small impurities from air, glass or elsewhere, of any relative importance. The amount of salt added should, out of regard to elimination of the diffusion potential and accuracy of measurement, not be too small, but must on the other hand be kept within reasonable limits, as our knowledge of the conditions in strong salt solutions is in many respects very vague. An excellent electrode liquid in our opinion, and one also frequently used by other investigators, is a solution of which one litre contains 0.01 equivalents of hydrochloric acid and 0.09 equivalents of potassium chloride (noted in the following as 0.01 n HCl + 0.09n KCl); naturally, however, many other similar mixtures may serve equally well.

The most important question of principle then is, how the concentration of hydrogen ions in such a mixture should be calculated.

Until a few years ago, this calculation was invariably based on determinations of conductivity in pure hydrochloric acid solutions, and on the view advanced by Sv. Arrhenius<sup>1)</sup> to the effect that the degree of dissociation of an acid when mixed with one of its salts was determined by the concentration of the common ion. This method, which is referred to in the following as the conductivity method, and which need not be described in detail here, was used for the calculation of  $\pi_0$ , in the investigations at this Laboratory published in 1909<sup>2)</sup>.

From the works published in recent years however, especially by English, American and Danish investigators, there can be no doubt that this method of calculating the degree of dissociation of an acid is incorrect, and that the problem should be treated according to the Lewis-Bjerrum activity theory.

According to N. Bjerrum<sup>3)</sup> the strong electrolytes in aqueous solutions must be regarded as completely dissociated, as the results arrived at by conductivity determinations and by osmotic or electrometric measurements, which might be, and have been

<sup>1)</sup> Zeitschr. physik. Chem. **31**, 204 (1899).

<sup>2)</sup> Compt.-rendus du Lab. Carlsberg **8**, 1 (1909).

<sup>3)</sup> Proc. of the 7 Intern. Congress of appl. Chem. London 1909, Section X; Det 16. skand. Naturforskermøde, Kristiania 1916, Section II; Zeitschr. f. Electrochem. **24**, 321 (1918); Meddelanden från K. Vetenskapsakademiens Nobel-institut, Bd. **5**, No. 16 (1919). See also Gilbert N. Lewis: Proc. Americ. Acad. **43**, 259 (1907); Zeitschr. physik. Chem. **61**, 129 (1907) and Journ. Americ. Chem. Soc. **34**, 1631 (1912).

interpreted as indicating incomplete dissociation of the electrolyte, are seen to have their natural explanation when the inter-ionic forces acting between the electrically charged ions are taken into consideration. Owing to these inter-ionic forces, the active mass of an ion, its activity ( $a$ ) cannot be taken as equal to its concentration ( $c$ ), the proper equation for these quantities being

$$a = f_a \cdot c,$$

where  $f_a$ , termed the activity coefficient, differs for the different ions, and varies for the individual ion with the nature and concentration of the other ions present. The magnitude of the activity coefficient is thus dependent on the effect of the inter-ionic forces on the ionic activity, just as the coefficient of conductivity  $f_u$  and the osmotic coefficient  $f_o$  depend on the effect of these forces on the conductivity and the osmotic pressure respectively. These coefficients however, are not of equal magnitude under like conditions, and it is therefore incorrect to take conductivity determinations as a basis for calculating the ionic activity, which in electrometric measurements is the factor which determines the potential.

We do not propose here to go further into the comprehensive literature of recent years on this subject, but will merely make brief mention of its importance for the question dealt with in the present work.

In a solution of the nature above described, 0.01 n HCl + 0.09 n KCl, we must, then, according to Bjerrum's view, regard both the hydrochloric acid and the potassium chloride as completely dissociated. The concentration of hydrogen ions  $c_H$  is therefore 0.01 n, but the quantity  $a_H$ , which determines the potential, is not 0.01 n, but  $0.01 n \times f_a$ , as

$$a_H = f_a \cdot c_H,$$

and the question now is, whether it is possible, and if so, by what means, to determine the magnitude of  $f_a$  in this and similar cases.

a) N. Bjerrum has, on the basis of his own and Milner's<sup>1)</sup> calculations and theoretical observations, and supported by the experimental material then extant, already, in 1916<sup>2)</sup>, established the following formula for calculating the activity coefficient:

$$-\log f_a = k \sqrt[3]{c} \quad (\text{III})$$

<sup>1)</sup> Phil. Mag. (6) **23**, 551 (1912); **25**, 743 (1913).

<sup>2)</sup> Meddelelser fra det 16. skand. Naturforskermode p. 226.

where  $f_a$  is the activity coefficient for the ion in question, that is, in the present instance, for the hydrogen ion, while  $c$  indicates the total concentration of ions. This formula can only be used with comparatively low concentrations of electrolytes, but Bjerrum has since rendered it applicable to higher concentrations of ions by adding a correction term approximately proportional to the ion concentration. This correction term, which gives a complete expression for the entire influence exerted by the ions present in addition to the above mentioned electric, inter-ionic effect, is formulated by Bjerrum, who lays special stress on the question as to hydration of the ion in question, as follows:

$$m \times \log \left( \frac{p}{p_0} \right),$$

where the proportion  $\frac{p}{p_0}$  indicates the relation between the vapour tension of the solution and that of pure water, while  $m$  denotes the number of water molecules in the hydrated ion, that is to say, in this case, in the hydrated hydrogen ion. Bjerrum's amended formula thus appears as follows:

$$-\log f_a = k \sqrt[3]{c} + m \log \left( \frac{p}{p_0} \right) \quad (\text{IV}).$$

At low concentrations of electrolytes, where  $p$  is very nearly equal to  $p_0$ , formula (IV) approaches very nearly to formula (III).

$\beta$ ) J. N. Bronsted<sup>1)</sup> has on the basis of extant experimental material, and supported by comprehensive experiments made by himself and his collaborators with the solubility of sparingly soluble salts in weak salt solutions, given the following simple formula for calculating the activity coefficients in highly diluted "ideal" electrolyte solutions:

$$\ln f_a = -3 a \sqrt{c} \quad (\text{V})$$

where  $c$  denotes total concentration of ions. In such diluted solutions, it is possible to disregard the size and specific qualities of the ions, and the magnitude of  $a$  is therefore determined solely by the actual "Milner effect", so that  $a$  has the same value for all monovalent ions.

Even at ion concentrations between 0.001 and 0.01 n however, this simple formula is no longer applicable, and it has been found that the deviation from the formula is of different magnitude for the different electrolytes. Bronsted therefore,

<sup>1)</sup> Journ. Americ. Chem. Soc. **44**, 942 (1922).

in calculating the activity coefficient for electrolyte solutions of medium strength, makes use of a more complicated formula, which in addition to the term  $3\alpha\sqrt{c}$  contains a correctional term proportional to the concentration:

$$\ln f_a = -3\alpha\sqrt{c} - k \cdot c \text{ (VI).}$$

In this formula, which can be used for solutions up to 0.1 n and a little over,  $k$  is a constant, the magnitude of which depends on various characteristic conditions for each separate ion, first and foremost the ion hydration and ion magnitude, so that  $k$  differs in value for the different ions. As the electrolyte concentration diminishes, the correctional term will be of less and less importance, so that the formula VI passes gradually over into formula V.

With monovalent electrolytes,  $\alpha$  has the value of 0.32, and formula (VI) can therefore, after dividing by 2.303, be written as follows:

$$-\log f_a = 0.417\sqrt{c} - \beta \cdot c \text{ (VII).}$$

In this connection, we should further mention that P. Debye and E. Hückel<sup>1)</sup> in a paper published last year, have worked out a theory based on purely theoretical considerations, as to the conditions which prevail in highly diluted solutions of electrolytes. The calculation of Debye and Hückel lead to equations of precisely the same kind as those Brønsted arrived at by experimental processes. Brønsted, for instance, in his latest work, on coefficients of ion activity, published in collaboration with Victor K. La Mer<sup>2)</sup> calculates that the value for  $\alpha$  which, as mentioned above, he had given as 0.32, must, according to Debye and Hückel, be 0.38, and he points out that the experimental results noted in the mentioned work agree at least as well with the theoretical values for  $\alpha$  as with the earlier experimental results.

γ) A number of American investigators<sup>3)</sup> have during the past decade produced very valuable contributions, both theoretic-

<sup>1)</sup> Physik. Zeitschr. **24**, 185 (1923).

<sup>2)</sup> Journal. Americ. Chem. Soc. **46**, 555 (1924)

<sup>3)</sup> James H. Ellis: Journ. Americ. Chem. Soc. **38**, 737 (1916); Arthur A. Noyes and James H. Ellis: *ibid.* **39**, 2532 (1917); Herbert S. Harned: *ibid.* **37**, 2460 (1915), **38**, 1986 (1916), **42**, 1808 (1920), **44**, 252 (1922); Duncan A. Mac Innes: *ibid.* **41**, 1086 (1919), G. A. Linhart: *ibid.* **41**, 1175 (1919); Arthur A. Noyes og Duncan A. Mac Innes: *ibid.* **42**, 239 (1920); Ming Chow: *ibid.* **42**, 488 (1920); Gilbert N. Lewis and Merle

tical and experimental, to the solution of the question here referred to. Herbert S. Harned for instance, has already, in 1920, (l. c.), set up the following formula for calculation of the activity coefficients of electrolyte solutions:

$$\log f_a = a \cdot c - \beta c^m \quad (\text{VIII}).$$

This formula is, it is true, of precisely the same character as that used by Bronsted (Formula VI, p. 24) but while the latter contains a constant common to all electrolytes, and only one quantity varying with the nature of the electrolyte, Harned's formula has no fewer than three quantities varying with the nature of the electrolyte,  $a$ ,  $\beta$  and  $m$ , all of which are determined experimentally. Obviously, it is easier to obtain agreement between calculated values and those actually found, the more experimentally determined constants we introduce into the formula used for calculation, and Harned's formula can therefore be used for a somewhat wider field of concentrations than Bronsted's. In the case of mixtures of hydrochloric acid and a chloride at fairly high concentrations, however, even Harned's formula will not serve. In such cases, Harned and Brumbaugh (l. c. 1922) suggest the addition of a correctional term, making the formula appear as follows:

$$\log f_a = a c, - \beta \mu^m + a'' (\mu - c), \quad (\text{IX})$$

and where the concentration of hydrochloric acid is very slight, a further correctional term is added, giving the formula as

$$\log f_a = a c, - \beta \mu^m + a'' (\mu - c) + \gamma \left( \frac{\mu - c}{c} \right) \quad (\text{X}).$$

In these formulæ,  $a$ ,  $\beta$ ,  $m$ ,  $a''$  and  $\gamma$  are experimentally determined constants, which, however, in the case of  $a$ ,  $\beta$  and  $m$  differ in value for the different chlorides,  $c$ , denotes the concentration of the hydrochloric acid, while  $\mu$  gives the concentration expression "ionic strength" introduced by Lewis and Randall (l. c. 1921) which takes into consideration, not only the concentration of ions, but also the ion valency<sup>1</sup>).

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Randall: *ibid.* **43**, 1112 (1921); Herbert S. Harned and Norman J. Brumbaugh: *ibid.* **44**, 2729 (1922); M. Knobel: *ibid.* **45**, 70 (1923); J. N. Pearce and A. R. Fortsch: *ibid.* **45**, 2852 (1923); R. H. Dalton, R. Pomeroy and L. E. Weymouth: *ibid.* **46**, 60 (1924).

<sup>1</sup>) See also: J. N. Bronsted and Victor K. La Mer: *Journ. Americ. Chem. Soc.* **46**, 558 (1924).

The introduction of so many experimentally determined constants into a formula naturally restricts its value to an essential degree, though the field of concentration within which it may be used is naturally extended.

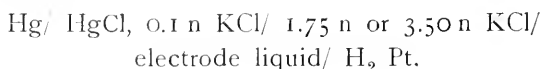
Finally, we would merely call to mind that in the measurement and calculation of activity coefficients, it is generally a question of the activity coefficient of the acid or the salt ( $f_a$ ) and not the coefficients of activity of the ions ( $f'_a$  and  $f''_a$ ). These quantities are connected by the equation

$$f_a^2 = f'_a \cdot f''_a,$$

but we have here no sure means at present — especially owing to the lack of any method for complete elimination of the diffusion potential — of determining the activity coefficient of the separate ions. Add to this the important question, emphasised especially by J. N. Brønsted<sup>1)</sup> as to specific interaction of ions, and we must agree with Lewis and Randall (l. c. (1921)) that »At the present time, we must conclude that the determination of the absolute activity of the ions is an interesting problem, but one which is yet unsolved«.

### *Experimental Results.*

The experimental results collected in Tables 4, 5, 6 and 7 were all obtained by measuring elements of the following composition:



where the electrode liquid consisted of dilute hydrochloric acid, containing potassium- or sodium chloride in more or less considerable quantity.

The calomel electrode used for the measurements was corrected by daily measurements with several comparatively freshly prepared calomel electrodes showing very close agreement one with another, these being thus used as standard electrodes.

For elimination of the diffusion potential, measurements were made both with 1.75 n and with 3.50 n potassium chloride solution as connecting liquid, the extrapolated value for the electromotive force of the cell,  $\pi_{\text{extrap}}$ , being then found as before

<sup>1)</sup> Journ. Americ. Chem. Soc. **44**, 877 (1922), **45**, 2898 (1923).



(see p. 17). The contact surface between the connecting and electrode liquids was made, in the main experiments, shown in Table 4, partly by ordinary dipping, partly by flowing junction (see p. 16); in the experiments shown in Tables 5, 6 and 7, ordinary dipping was used throughout.

All measurements were made at 18° C., the electrodes being placed in a well-regulated water thermostat, the connecting liquid being placed outside it; for the rest, the measurements were carried out as mentioned in Section A (p. 5). All measurement results have been corrected by the usual correction equation, so that the values noted for  $\pi_{\text{extrap}}$  apply to a pressure of 1 atmosphere dry hydrogen.

Apart from the main experiments, which, as mentioned, are shown in Table 4, and comprise the standardising material proper, we have, also in order to get a view of the influence of the potassium chloride and sodium chloride on the measurement results, investigated a series of solutions, partly with varying concentration of hydrochloric acid and constant concentration of potassium chloride (Table 5), partly with constant hydrochloric acid concentration and varying concentration of potassium- or sodium chloride respectively (Tables 6 and 7).

The tables, which need no further explanation, show that the calculation of  $\pi_0$  was made

a) from determination of conductivity.

$\beta$ ) after N. Bjerrum:  $-\log f_a = 0.2 \sqrt[3]{c} \pm m \log \left( \frac{P}{P_0} \right)$

1) for  $m = 0$

and 2) for  $m = 11$  (potassium chloride solutions)

$m = 16$  (sodium — — )

or  $\gamma$ ) after J. N. Bronsted:  $-\log f_a = 0.417 \sqrt{c} - \beta \cdot c$

for  $\beta = 0.40$  (potassium chloride solutions)

for  $\beta = 0.48$  (sodium — — ).

a) When  $\pi_0$  is calculated from determinations of conductivity, the main experiments in which the total concentration of ions was throughout 0.1 n, give the value

$\pi_0 = 0.3380$  volts (for the potassium chloride experiments)

$\pi_0 = 0.3374$  volts (for the sodium chloride experiments).

In calculating the mean values, we have here disregarded the most acid solutions, where the high value of 1.17 indicated

TABLE 4.  
MEASUREMENTS OF CHAINS:

Hg | HgCl, 0.1 n KCl | 1.75 n or 3.50 n KCl | Electrode liquid | H<sub>2</sub>, Pt.  
Electrode liquid with constant electrolyte concentration, 0.1 n (HCl + KCl or HCl + NaCl).

Number of experiments using.	Electrode liquid	$\tau_0$ (calculated from measurements of conductivity)	$\tau_0$ (calculated after <i>N. Bjerrum</i> ) $\left(-\log f_a = 0.2 \log \frac{1}{S \cdot f_a} + m \log \left(\frac{p}{p_0}\right)\right)$	$\tau_0$ (calculated after <i>J. N. Brønsted</i> ) $\left(-\log f_a = 0.417 \log \frac{1}{S \cdot f_a} - \beta c\right)$								
					$\tau_0$ Volt	$\tau_0$ Volt	$\tau_0$ Volt					
ordinary » dipping »	» flowing junction »	0.0577 $\times \log \frac{1}{S \cdot a}$	0.0577 $\times \log \frac{1}{S \cdot f_a}$	0.0577 $\times \log \frac{1}{S \cdot f_a}$								
2	1	0.002 n HCl + 0.098 n KCl	0.06	0.4963	0.1579	0.3384	0.1611	0.3352	0.1602	0.3361	0.1610	0.3353
2	1	» + 0.095 »	0.27	0.4731	0.1350	0.3381	0.1381	0.3350	0.1372	0.3359	0.1381	0.3350
36	6	0.010 » + 0.090 »	0.64	0.4556	0.1176	0.3380	0.1208	0.3348	0.1198	0.3358	0.1207	0.3349
	2	» + 0.080 »	1.08	0.4381	0.1002	0.3379	0.1034	0.3347	0.1025	0.3356	0.1033	0.3348
	2	» + 0.070 »	1.60	0.4280	0.0901	0.3379	0.0932	0.3348	0.0923	0.3357	0.0932	0.3348
2	2	» + 0.060 »	2.10	0.4208	0.0828	0.3380	0.0860	0.3348	0.0851	0.3357	0.0860	0.3348
	3	» + 0.050 »	2.46	0.4152	0.0773	0.3379	0.0804	0.3348	0.0795	0.3357	0.0804	0.3348
	2	» + 0.040 »	2.90	0.4107	0.0727	0.3380	0.0759	0.3348	0.0749	0.3358	0.0758	0.3349
	2	» + 0.020 »	3.65	0.4030	0.0655	0.3375	0.0687	0.3343	0.0677	0.3353	0.0686	0.3344
3	5	» + 0 »	4.34	0.3968	0.0599	0.3369	0.0631	0.3337	0.0621	0.3347	0.0630	0.3338
2	2	0.002 n HCl + 0.098 n NaCl	± 0.03	0.4953	0.1579	0.3374	0.1611	0.3342	0.1597	0.3356	0.1606	0.3347
2	3	» + 0.095 »	± 0.09	0.4724	0.1350	0.3374	0.1381	0.3343	0.1368	0.3356	0.1376	0.3348
4	1	0.010 » + 0.090 »	0.34	0.4550	0.1176	0.3374	0.1208	0.3342	0.1194	0.3356	0.1202	0.3348





TABLE 7.

## MEASUREMENTS OF CHAINS:

Hg | HgCl, 0.1 n KCl | 1.75 n or 3.50 n KCl | Electrode liquid | H<sub>2</sub> Pt.

Electrode liquid with constant concentration of hydrochloric acid and with varying sodium chloride concentration.

Electrode liquid	I.7 Milli- Volts	$\pi_{\text{extrap}}$ Volts	$\pi_0$ (calculated from measurements of conductivity)		$\pi_0$ (calculated after <i>N. Bjerrum</i> ) $(-\log f_a = 0.2 \sqrt{c} + m \log \frac{p}{p_0})$		$\pi_0$ (calculated after <i>J. N. Brønsted</i> ) $(-\log f_a = 0.417) c = 0.48 c$
			$0.0577 \frac{I}{\log S \cdot a}$	$\pi_0$ Volt	$0.0577 \frac{I}{\log S \cdot f_a}$	$\pi_0$ Volt	
0.003 n HCl + 0.1 n NaCl	0	0.4849	0.1478	0.3371	0.1510	0.3339	0.1504
» + 0.4 »	-0.5	0.4837	0.1492	0.3345	0.1541	0.3296	0.1497
» + 1.2 »	-1.2	0.4767	0.1525	0.3242	0.1578	0.3189	0.1387
» + 2.0 »	-1.5	0.4682	0.1559	0.3123	0.1601	0.3081	0.1242
0.005 n HCl + 0.08 n NaCl	+0.5	0.4723	0.1348	0.3375	0.1378	0.3345	0.1374
» + 0.095 »	+0.09	0.4724	0.1350	0.3374	0.1381	0.3343	0.1376
» + 0.2 »	-0.2	0.4734	0.1357	0.3377	0.1396	0.3338	0.1380
» + 0.4 »	-0.7	0.4717	0.1364	0.3353	0.1413	0.3304	0.1369
» + 0.8 »	-0.9	0.4682	0.1381	0.3301	0.1435	0.3247	0.1321
» + 1.2 »	-1.3	0.4643	0.1397	0.3246	0.1451	0.3192	0.1258
» + 1.6 »	-1.4	0.4601	0.1413	0.3188	0.1463	0.3138	0.1188
» + 2.0 »	-1.7	0.4559	0.1431	0.3128	0.1473	0.3086	0.1113
							0.3345
							0.3340
							0.3380
							0.3362
							0.3353
							0.3356
							0.3366
							0.3357
							0.3348
							0.3301
							0.3385
							0.3413
							0.3446

that the elimination of the diffusion potential was uncertain. On going through Tables 5, 6 and 7, with a glance at Fig. 2, where the value of  $\pi_0$  is taken as ordinate, and the total ion concentration as abscissa, it will be seen that the value for  $\pi_0$ , so calculated decreased with increasing salt concentration. It will be seen from the curves that the descent is greater in the case of sodium chloride than in that of potassium chloride solution, and in both cases very nearly proportional to the concentration of salt. In slight concentrations of salt, the curves turn off to something approaching a course parallel to the abscissa, finally intersecting the ordinate axis at the same point, answering to  $\pi_0 = 0.3382$  volts for a non-saline solution, which agrees well with the value  $\pi_0 = 0.3380$  volts found above for potassium chloride solutions.

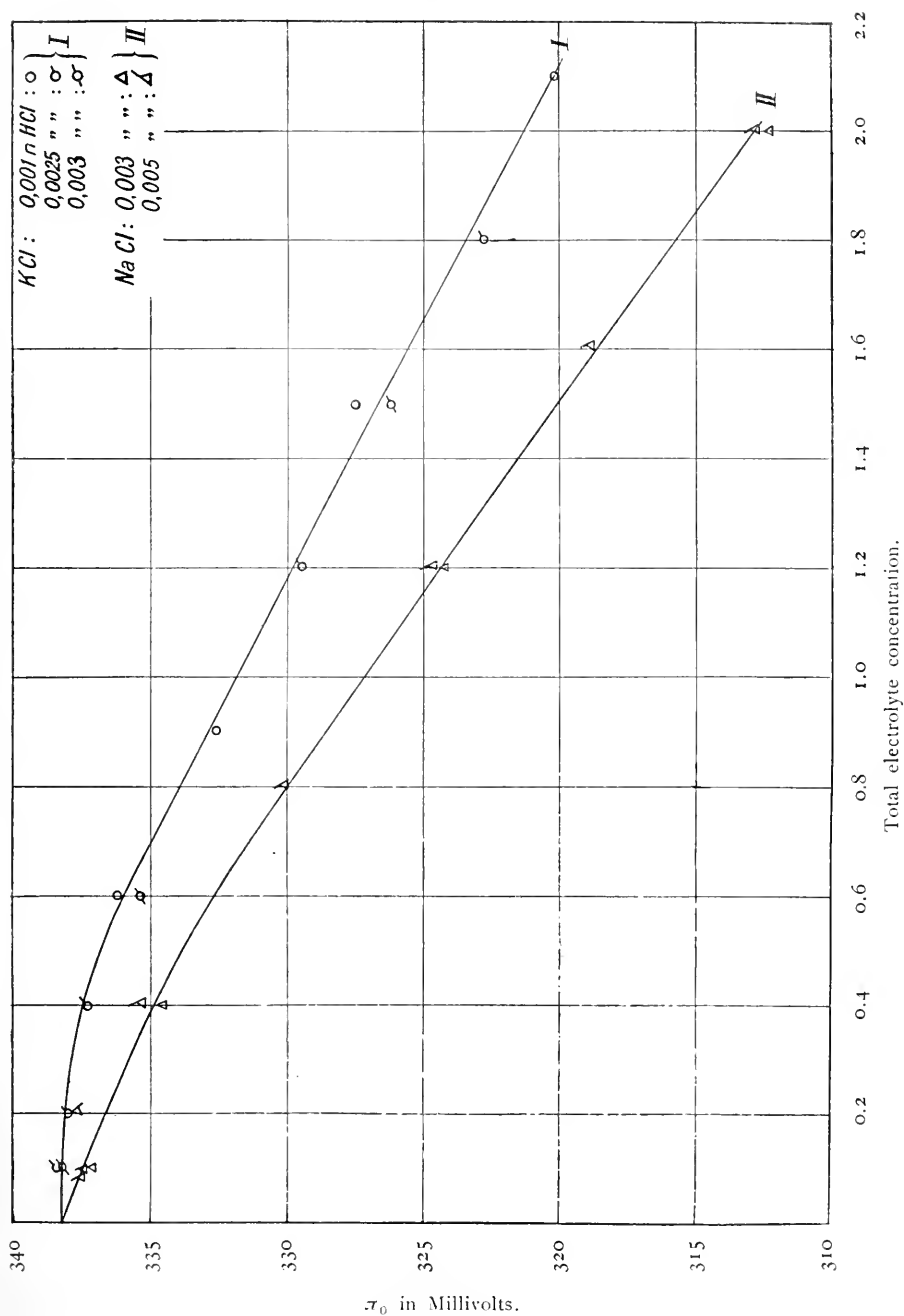
The experimental results obtained distinctly show that this method of calculating  $\pi_0$  involves one or more sources of error. For, if the concentration of hydrogen ions were really the determining factor in the potential, and if the degree of dissociation of the acid were really determinable by this method, we should, of course, get the same value for  $\pi_0$  in all experiments, always assuming the measurements to be correct and the elimination of the diffusion potential complete. The values found for  $\pi_0$  further show, that these sources of error differ in importance according as potassium- or sodium chloride is used, and that this difference is apparent even at such slight concentration as 0.1 n.

In spite of this, we must, with Warren C. Vosburgh and Marion Eppley<sup>1)</sup> agree with W. M. Clark's proposal to retain this mode of calculating  $\pi_0$  for the present. For the question as to calculation of the coefficient of activity is, as mentioned above, (p. 26) not yet solved, and we therefore consider it best, save in exceptional cases to maintain the continuity in order to avoid confusion. On the other hand, we are quite aware that the standardisation of  $\pi_0$  will henceforward call for a somewhat more detailed definition than that hitherto employed.

The majority of extant measurements of concentration of hydrogen ions, at any rate in biochemical literature, have been calculated with a value for  $\pi_0$  at 18° of 0.3380 or 0.3377 volts, ac-

<sup>1)</sup> Review of "The Determination of Hydrogen Ions" (by W. Mansfield Clark) Second edition: Journ. Americ. Chem. Soc. **45**, 3145 (1923).

Fig. 2.



cording as pressure was reckoned as 1 atmosphere dry or moist hydrogen. Also, it is just this value for  $\pi_0$  that is obtained when the composition of the electrode liquid used for standardisation does not differ essentially from that used not only by ourselves but also by many other investigators, viz. the standard solution „0.01 n HCl + 0.09 n KCl” and when the measurement is carried out in the manner above described (by flowing junction or by ordinary dipping, with measurement taken immediately after, and extrapolation after N. Bjerrum). We therefore consider it advisable to retain the above-mentioned value of 0.3380 volts as the standard value for  $\pi_0$  and to include the mentioned metritechnical details in the definition of this quantity. We likewise consider it advisable, to avoid misunderstanding and confusion, that the terms  $C_H$  and  $p_H$  should only be used in the sense hitherto ascribed to them.

It goes without saying that the measurement of concentration of hydrogen ions in a liquid to be investigated must be carried out in the manner above described when the standard value of 0.3380 volts is used for calculating the concentration of hydrogen ions in the liquid or the hydrogen ion exponent, on the basis of measurement results.

Finally, we should point out that the measurements on the basis of which the value  $\pi_0 = 0.3380$  (or 0.3377 as the case may be) was suggested some time back by this laboratory<sup>1)</sup> as the standard value for electrometrical measurements of hydrogen ions, were made with mixtures of hydrochloric acid and sodium chloride, and should thus, in accordance with what has now been found, have given a value for  $\pi_0$  about 0.6 millivolt lower. That these earlier measurements of ours gave somewhat too high a value for  $\pi_0$  is probably due to inadequacy of the method employed, (the values found for  $\pi_7$  at that time were somewhat higher than now). That the mean value found for  $\pi_0$  was precisely 0.3380 (or 0.3377) volts is purely accidental.

$\beta)$  When  $\pi_0$  is calculated according to N. Bjerrum's simple formula (see p. 22)

$$-\log f_a = 0.2 \frac{3}{4} c,$$

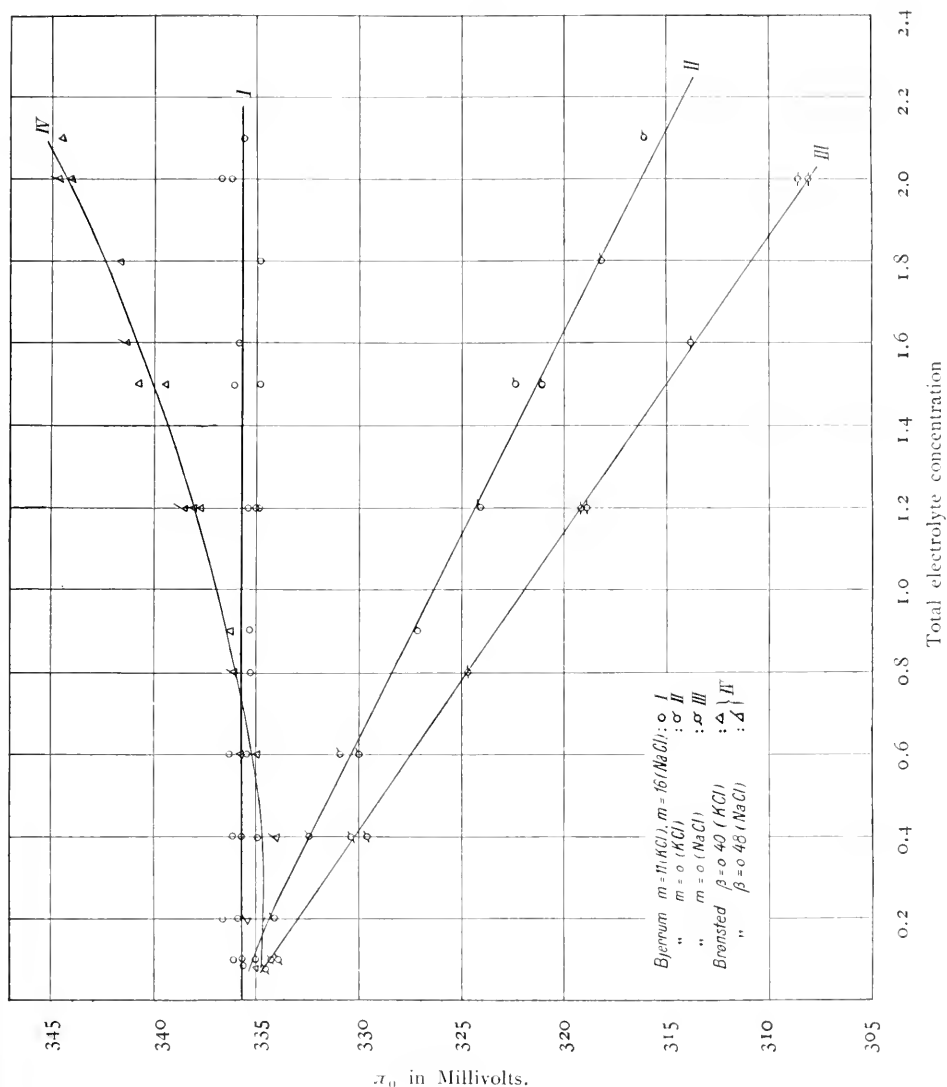
the main experiments with potassium chloride solution give  $\pi_0 = 0.3348$ , and with sodium chloride solutions  $\pi_0 = 0.3342$ . Tables

<sup>1)</sup> Comptes-rendus du Lab. Carlsberg **8**, 24 (1909); Ergebnisse der Physiologie **12** 418 (1912).



5, 6 and 7, however, and especially the graphical reproduction in Fig. 3 (Curves II and III) show that the value for  $\pi_0$  de-

Fig. 3.



creases greatly with increasing concentration of salt. The linear correction term in Bjerrum's amended formula (see p. 23)

$$-\log f_a = 0.2 \sqrt{c} + m \log \left( \frac{p}{p_0} \right),$$

counteracts this decrease when  $m$  is given a suitable value (11 for potassium- and 16 for sodium chloride solutions)<sup>1)</sup>. When this last formula is used for calculation of  $\pi_0$ , then both the main experiments and the others, potassium- as well as sodium chloride experiments, give very nearly the same value for  $\pi_0$ , the mean of all experiments being 0.3357 volts. In Fig. 3, Curve I, the values found are seen to range themselves evenly about a line parallel with the abscissa axis through the ordinate 335.7, and it will be seen from the figure that curves II and III also, as was to be expected, will intersect the ordinate axis very near this point. It will therefore, we think, be natural when employing Bjerrum's mode of calculating the activity of hydrogen ions and the coefficient of activity, to use the more complicated formula for  $\log f_a$  and take 0.3357 volts as the value for  $\pi_0$ .

For the reasons given above (see p. 34) it would seem premature at present to introduce the activity principle in place of the concentration principle for measurements of hydrogen ions in biochemical investigations generally; on the other hand, it must be borne in mind that measurements of hydrogen ions in salt solutions of any considerable strength often give results which can hardly be explained according to the old view.<sup>2)</sup> In cases where it is a question not only of reproducing a measurement result, calculated in a manner generally accepted, but also of using the result arrived at in some other way, as for instance for further calculations, it will therefore be necessary to use the Lewis-Bjerrum activity principle, at any rate on terms of equality with the old concentration principle. It would then be advisable, in order to avoid confusion, that a sharp distinction should be made between activity and concentration in the terms employed. We therefore suggest, that in accordance with the practice of

1) The value  $m = 11$  for potassium chloride solutions agrees well with the measurements made by E. Schreiner (*Zeitschr. f. anorg. u. allgem. Chemie* **121**, 330 (1922)) when the measurement results are corrected for the "salt error" of the quinhydrone electrode (S. P. L. Sørensen, Margrethe Høyrup and K. Linderstrøm-Lang: *Comptes-rendus du Lab. Carlsberg* **14**, no. 14 (1921) and K. Linderstrøm-Lang *ibid.* **15**, no. 4 (1923)).

2) We would here merely call to mind that the concentration of hydrogen ions found in a mixture of diluted hydrochloric acid and abundant chloride by electrometric measurement and calculation according to the old method can be considerably higher than would correspond to the complete dissociation of the acid, whereas according to the old view, it should be considerable lower, owing to the presence of the salt.

Bjerrum and his collaborators, the terms  $c_H$ ,  $p_H$  and  $\pi_0$  should be allowed to retain their original significance,  $a_H$ ,  $pa_H$  and  $a\pi_0$  being used to denote respectively hydrogen ion activity, exponent of hydrogen ion activity ( $pa_H = -\log a_H$ ) and the  $\pi_0$  used in calculating the activity of hydrogen ions, the value of which we suggest should be fixed at 0.3357 volts.

We would point out that this last suggestion is not in accordance with the method hitherto followed by Bjerrum and his collaborators<sup>1)</sup>, who have used the somewhat lower value for  $a\pi_0$  found by the simple cube root formula. We think, however, that since the linear correction term in Bjerrum's formula cannot be dispensed with in higher salt concentrations, it should also be taken into consideration in calculating the value for  $a\pi_0$ .

$\pi_0$  (0.3380 volts) will then be 0.0023 volts higher than  $a\pi_0$  (0.3357 volts), from which again it follows that the  $p_H$  determined by a measurement of hydrogen ions will be abt. 0.04 below the corresponding  $pa_H$ , as, (at 18°)

$$p_H = \frac{\pi - \pi_0}{0.0577} = \frac{\pi - (a\pi_0 + 0.0023)}{0.0577} = pa_H - 0.04$$

$\gamma$ ) When  $\pi_0$  is calculated according to J. N. Brønsted's formula (see p. 24)

$$-\log f_a = 0.4171 \sqrt{c} - \beta c,$$

and  $\beta$  is given suitable values (0.40 for potassium- and 0.48 for sodium chloride solutions), the main experiments (Table 4) give very nearly the same value for  $\pi_0$  (0.3348 volts) as by Bjerrum's cube root formula. Tables 5, 6 and 7 as well as Fig. 3, curve IV, however, show that while this value for  $\pi_0$  remains more or less constant, as long as the electrolyte concentration does not exceed 0.5–0.6 n, a further increase in the electrolyte concentration will occasion an increase of  $\pi_0$  which cannot be expressed as a linear function of the concentration.

This formula, which Brønsted, as already noted, (p. 24) only intended for use with weak and medium electrolytic solutions, is thus not so well suited as Bjerrum's expanded formula for the calculation of activity coefficient when dealing with electrolyte solutions of any strength.

<sup>1)</sup> N. Bjerrum og J. K. Gjaldhæk: Den Kgl. Vet. og Landbohøjskoles Aarskrift 1919 p. 73; Carl Faurholt: ibid. 1924 p. 20; se ogsaa E. Schreiner: Zeitschr. für anorg. und allgem. Chemie. **115**, 190 (1921), **121**, 332 (1922) and E. J. Warburg: The Biochemical Journal **16**, 166 (1922).

An amendment of the formula in accordance with the calculations of Debye & Hückel (see p. 24) does not render it more adapted to high salt concentrations.

#### D. Proposals for Standardisation.

It is of course important that in the determination, calculation and publication of measurements of hydrogen ions, the same rules should, as far as possible, be observed. We have therefore considered it advisable to put forward the following proposal for standardisation of nomenclature and methods. The proposal is based on the experimental material hitherto published, including the experiments dealt with in the present paper, and follows in all essentials the lines of the proposal made by W. M. Clark, quoted above (p. 2).

1) In electrometric measurements of hydrogen ions, a sharp distinction should be made between concentration and activity of the hydrogen ions.

2) In statements of concentration of hydrogen ions, the terms  $c_H$ ,  $p_H$  and  $\pi_0$  should be used, retaining the same significance as hitherto.

3) In stating the activity of hydrogen ions, the terms  $a_H$ ,  $pa_H$  and  $a\pi_0$  should be used, indicating respectively activity, exponent of activity, and the  $\pi_0$  used in calculating the activity of hydrogen ions.

4) The standard value of  $\pi_0$  at  $18^\circ$  should be fixed at 0.3380 volts and defined as the electromotive force of a cell of which the left half consists of a 0.1 n potassium chloride-calomel-mercury electrode, the right half consisting of a hydrogen-platinum electrode in an electrode liquid having a concentration of hydrogen ions of 1 normal, at  $18^\circ$  and with a hydrogen pressure of 1 atmosphere dry hydrogen, the diffusion potential being taken as eliminated.

5) The standard value for  $a\pi_0$  at  $18^\circ$  to be fixed at 0.3357 volts and defined as the electromotive force of a cell composed as indicated under 4), save that the electrode liquid here has a 1 n activity of hydrogen ions, not the corresponding concentration of the same.

6) The variation in value of  $\pi_0$  according to temperature to be fixed, in accordance with W. M. Clark's proposal, as follows:

Temperature..	15°	18°	20°	25°	30°	37.5°	40°
$\pi_0$ . . . . .	0.3382	0.3380	0.3379	0.3376	0.3373	0.3364	0.3360

For the variation of  $a\pi_0$  with the temperature, it is suggested that for the present, corresponding values should be used.

7) Where measurements are made with a 1 n- or a 3.5 n-potassium chloride-calomel electrode in place of the 0.1 calomel electrode, the measurement should be referred to the standard electrode by adding the additional term  $a$  noted in Table 3 (p. 12) for the temperature in question, the standard value for  $\pi_0$  at such temperature being then used in calculating  $p_H$  or  $pa_H$ .

The saturated potassium chloride-calomel electrode should never be used as a standard electrode, but only as a working electrode, its potential being checked immediately before or after the measurement by measuring it against an electrode of known potential.

8) Where a quinhydrone electrode is used for the measurement, in place of the ordinary hydrogen electrode, the electromotive force of a cell like that given on p. 19 (as for the sign see p. 40) should be increased by + 0.7048 volts — and a correction may also have to be made owing to the electrolyte content of the electrode liquid, after which the usual formulae for calculation can be employed.

9) For elimination of the diffusion potential, it is proposed that Bjerrum's extrapolation method should be used, stating the magnitude of  $1\pi$  or at any rate indicating what method of elimination has been employed.

10) In specially accurate measurements, more than one calomel electrode should be used, and the constancy and durability of these should be tested from time to time against freshly prepared electrodes of the same sort, or against electrodes whose potential is known, preferably a hydrogen electrode in the electrode liquid »0.01 n HCl + 0.09 n KCl«. In the latter case, the calomel electrode is reckoned as being correct when  $\pi_{\text{extrap}}$  is 0.4556 volts, that is to say, when it is 0.1 n potassium chloride-calomel electrode.

11) Finally, we would offer the suggestion that the formulae for

the cells measured should be stated in accordance with the rules laid down by "Die Deutsche Bunsen Gesellschaft", where a positive sign before the measured difference of potential indicates the half cell to the left in the formula as the positive pole, i. e. the point where the positive current of the working cell issues from the latter.<sup>1)</sup> We would expressly point out that this mode of formulation is not always employed: G. N. Lewis, T. B. Brighton and R. L. Sebastian<sup>2)</sup> for instance, give exactly the opposite definition: „When E is given as positive, it indicates that the tendency of the positive current in the cell is to pass from left to right; when negative, from right to left". The one mode of formulation may be just as good as the other, but as it is not always stated, and cannot always be seen from the quantities given, which of the two modes has been employed, the lack of uniformity in formulation may give rise to misunderstanding, e. g. in temperature corrections, and we therefore propose that the method suggested by the Bunsen Gesellschaft should always be used, as it has been in the present paper.

<sup>1)</sup> R. Abegg, Fr. Auerbach, R. Luther: Messungen elektromotorischen Kräfte galvanischer Ketten p. 114 (1910).

<sup>2)</sup> Journ. Americ. Chem. Soc. **39**, 2255 (1917).

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# ON THE IONISATION OF PROTEINS

BY

K. LINDERSTRØM-LANG.

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It is a well known fact that the theory of adsorption, which has contributed so greatly to the increase and extension of our knowledge of the reciprocal reactions between substances, and of the conditions which determine the coexistence of phases, has in course of time been applied to the treatment of problems so widely dissimilar as inevitably to raise questions under this head which should have been taken up on a far broader basis. I am here refering to the question of proteins.

Wherever the adsorption theory has been applied, the formula «alterations of concentration in the boundary surface» is used to describe the association phenomena which occur when particles of one medium are suspended in another. It is impossible, however, to overlook the fact that such a view involves a certain partiality, due as far as I can see, to the empiric thermodynamic origin of the theory itself. Such a view is really meaningless in a physical sense unless the particles are of such magnitude that each is in phase-equilibrium with the dispersion medium and has a surface in the true sense of the word. This, however, is not the case with protein substances for, if we follow S. P. L. Sørensen<sup>1)</sup> and take the molecular magnitude of the egg albumin particles as 35000, each particle will contain abt. 3000 atoms, the presumable surface only abt. 1500, which, in the first place is one third of all the atoms in the particle, and secondly, of course, too small a number to form a surface in the ordinary sense.

It is however, often somewhat difficult to understand the

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<sup>1)</sup> Studies on Protein p. 356. Compt. rendus du Lab. Carlsberg **12**, 1917.

sharp distinction drawn between colloid chemists on the one hand and «ordinary» chemists on the other, seeing that their respective fields of investigation do after all undoubtedly run over continuously one into the other. It is perfectly natural, for instance that we should, with a system such as a protein solution, treated theoretically, arrive at the same result whether we start from the laws which determine the state of the molecules, and correctly draw the conclusion of an increase in the molecular magnitude up to the magnitude of the protein complexes, or start from the laws prevailing at the surface between large particles and the dispersion medium, correctly drawing the conclusion of a decrease of the particles down to the magnitude of the protein complexes.

It seems strange, therefore, that this continuity should not as a rule be properly observed, and it is this fact which has led to the publication of the present paper. In dealing with such a problem, it is impossible to avoid associating such a law as that of mass action closely with the adsorption isotherms of one sort or another, if we imagine the prevailing forces as of such a character as to give rise to a real association even in molecular-disperse solutions. We shall see, for instance, in the following, both the law of mass action and a simple law of adsorption applied to the ionisation of certain particles in an aqueous solution, whereby the continuity above referred to will be plainly apparent.

In Section A, an ion adsorption theory put forward by Debye and Hückel<sup>1)</sup> will be described, this being taken as the basis of my subsequent observations. Section B gives the formulation of an expression for ion adsorption, based chiefly on the theories of Helmholtz and Debye for electric double layers and on chemical and as a matter of fact purely statistical considerations. Section C shows the continuity between this adsorption theory and expressions arrived at by the application of the law of mass action on particles of the adsorbent substance selected in Section B, the hydrogen ion being chosen as the ion adsorbed. And finally, Section D compares the results arrived at with those of experience.

I take this opportunity of expressing my thanks to Prof.

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<sup>1)</sup> Physik. Zeitschr. **24**, 185. (1923).

S. P. L. Sørensen, for his frequent encouragement and kindly interest in my work. I also wish to thank Lektor J. A. Christiansen for valuable advice in the final composition of the present paper.

### A. The Debye-Hückel Theory.

Since Milner<sup>1)</sup> in 1912—13 published his well-known investigation of the influence of ionic forces on the condition of electrolyte solutions, and Bjerrum<sup>2)</sup> established his theory of the complete dissociation of strong electrolytes, the progress made in this field has been chiefly imperical, until Debye and Hückel (loc. cit.) and later Debye<sup>3)</sup> succeeded, in an extremely simple fashion, in advancing the question as to influence of interionic forces on the osmotic pressure of the ions and their activity as far as could possibly be done on the basis on which our modern views as to ion solutions rest. Whether the problem can be regarded as solved even for highly diluted electrolyte solutions it is difficult to say from the experimental material available; it seems, however, as if there were certain difficulties attending the theory in connection with the determination of dependence upon temperature for the osmotic coefficient and the activity coefficient, as the theory, so far as can be judged from the scanty measurements of the heat solution of electrolytes, gives values for the same which by no means agree with the experimental results. I shall not enter into these difficulties here, but merely observe, that the salient point is the alteration of the dielectricity constant with the temperature. I hope, by the way, to revert to this question in a later work.

The following is a brief resumé of Debye and Hückel's theory: In a diluted electrolyte solution of the volume  $V$ , there are, per cc,  $n_1$  ions of the valency 1, and  $n_2$  ions of the valency 2, etc.,  $n_i$  ions with the valency  $i$ , where  $i$  can be either positive or negative. Since the solution must be electrically neutral, we must have

$$\sum i \cdot n_i = 0 \quad (1)$$

We now proceed to consider a single ion,  $Z$ , which we imagine as in the shape of a sphere with the radius  $a$ , and

<sup>1)</sup> Phil. Mag. (6) **23** and **25** (1912, 1913).

<sup>2)</sup> Proc. of the 7 intern. Congress of appl. Chem. London 1909, Section X; Det 16. skandinaviske Naturforskersmøde, Kristiania 1916, Sektion II; Zeitschr. f. Elektrochem. **24**, 321 (1918).

<sup>3)</sup> Physikalische Zeitschr. **25** (1924).

having the valency  $z$ . This ion would then at a distance  $r$  from its centre, produce an electric potential  $\frac{\epsilon \cdot z}{r \cdot D}$  provided there were no other ions present. ( $\epsilon$  is the elementary quantum of electricity,  $4.77 \cdot 10^{-10}$  C. G. S. and  $D$  is the dielectricity constant). In the electrolyte solution, there will however, if we take  $z$  as positive, be more negative than positive ions in the vicinity of  $Z$ , owing to the forces between the ions, and the potential at a distance of  $r$  from  $Z$  will therefore, owing to this unequal distribution in the vicinity of  $Z$ , fall below that value. Taking now the term  $\varphi$  to denote the potential at a point  $P$  at the distance  $r$  from  $Z$ , whereby  $\varphi$  is to be regarded as a mean value for a long period of time, then the concentration of ions of the valency  $i$  at this point will be  $n_i \cdot e^{-\frac{i \cdot \varphi \cdot \epsilon}{kT}}$ , where  $k$  is Boltmann's constant and  $T$  the temperature. The total density of charge  $\varsigma$  at this point will therefore be

$$\varsigma = \sum \epsilon \cdot i \cdot n_i \left(1 - \frac{i \cdot \varphi \cdot \epsilon}{k \cdot T}\right)^1 = - \sum \frac{\epsilon^2 i^2 n_i}{kT} \varphi, \quad (2)$$

here taking into consideration (1).

The Poisson equation, however gives us:

$$\Delta \varphi = - \frac{4 \pi}{D} \cdot \varsigma, \quad (3)$$

and we thus obtain, by combining (2) and (3), the following differential equation for the determination of  $\varphi$ :

$$\Delta \varphi = \frac{4 \pi \epsilon^2}{DkT} \cdot \sum i^2 n_i \cdot \varphi = x^2 \varphi, \quad (4)$$

$x$  being determined by  $x^2 = \frac{4 \pi \epsilon^2}{DkT} \cdot \sum i^2 n_i$ . By integration we obtain:

$$\varphi = A \cdot \frac{e^{-xr}}{r}, \quad (5)$$

where  $A$  is a constant determined as follows:

We imagine the ionic charge of  $Z$  situate in the centre of the sphere with radius  $a$ , and surrounded by a dielectric with the dielectricity constant  $D$  equal to that of the water. The poten-

<sup>1)</sup>  $\frac{i \cdot \varphi \cdot \epsilon}{kT}$  being a small quantity.

tial in the interior of the ion is now composed of the potential from the charge in the centre and the potential from the unequal distribution of the ions outside  $Z$ , and must thus be of the following form:

$\varphi = \frac{\varepsilon \cdot Z}{r \cdot D} + K$ ,  $r$  being the distance from the centre inside the ionic sphere and  $K$  the potential of the surrounding ions. Outside the ion,  $\varphi$  is determined by (5) and at the boundary surface of the ion, both  $\varphi$  and  $\frac{d\varphi}{dr}$  must be continuous functions, i. e.

$$A \cdot \frac{e^{-xa}}{a} = \frac{\varepsilon \cdot Z}{a \cdot D} + K \quad (6)$$

and

$$A \cdot e^{-xa} \cdot \frac{1 + xa}{a^2} = \frac{\varepsilon \cdot Z}{D \cdot a^2}, \quad (7)$$

by means of which two equations  $A$  and  $K$  can be determined.

$$A = \frac{\varepsilon \cdot Z \cdot e^{xa}}{(1 + xa) D} \quad (8)$$

$$K = - \frac{Z \cdot \varepsilon \cdot x}{D (1 + xa)} \quad (9)$$

The potential  $\psi$  on the surface of the ionic sphere will then be:

$$\psi = \frac{Z \cdot \varepsilon}{D \cdot a} - \frac{Z \cdot \varepsilon \cdot x}{D (1 + xa)} \quad (10)$$

The quantity  $x = \sqrt{\frac{4\pi\varepsilon^2}{DkT} \sum n_i}$  plays a dominant part in the theory. It has the dimension of a reciprocal length, and is, according to Debye, and as we shall later see, the reciprocal distance between the layers in an electric double layer, (see p. 17).

In highly diluted solutions, where  $xa$  is small in comparison with 1, we have

$$\psi = \frac{Z \cdot \varepsilon}{D \cdot a} - \frac{Z \cdot \varepsilon \cdot x}{D}. \quad (11)$$

There is now a certain connection between  $\psi$  and the thermodynamic state of the solution, whereby the last term in (10) and (11) expressing the influence exerted by the surrounding ions on each separate ion, is closely connected with the de-

crease in free energy of the solution occasioned by the inter-ionic forces. We can therefore, thermodynamically, deduce the formula for  $f_0$ , the osmotic coefficient, and  $f$ , the activity coefficient for all ions together:

$$1 - f_0 = \frac{x}{6DkT \sum n_i} \sum \epsilon^2 i^2 n_i \cdot \frac{3 - 3\{x^2 a_i^2 - 2xa_i + 2\ln(1 + xa_i)\} \frac{(1 + xa_i)}{a_i^3 \cdot x^3}}{(1 + xa_i)} \quad (12)$$

$$\ln f = - \frac{x}{2DkT \sum n_i} \sum \epsilon^2 i^2 n_i \frac{1}{1 + xa_i}, \quad (13)$$

where  $a_i$  is the radius of the ion marked  $i$ . For the activity coefficient,  $f_i$ , of this ion, we have the simple expression:

$$\ln f_i = - \frac{x}{2DkT} \cdot \epsilon^2 i^2 \frac{1}{1 + xa_i}. \quad (14)$$

In highly diluted solutions, (12), (13) and (14) are transposed into:

$$1 - f_0 = \frac{x}{6DkT} \frac{\sum \epsilon^2 i^2 n_i}{\sum n_i} \quad (12a) \quad \ln f = - \frac{x}{2DkT} \frac{\sum \epsilon^2 i^2 n_i}{\sum n_i} \quad (13a)$$

$$\ln f_i = - \frac{x}{2DkT} \cdot \epsilon^2 i^2, \quad (14a)$$

i. e.

$$\sum n_i \ln f_i = \ln f \cdot \sum n_i. \quad (15)$$

For the derivation of the formulæ see Debye's last work, 1924 (loc. cit.).

## B. Ion Adsorption.

Given a pure solid substance  $S$  in stable form, in contact with a saturated aqueous solution of itself, it is a well known fact that the condition of equilibrium can be expressed as follows:

$$a_s = P_s$$

$a_s$  being the activity,  $P_s$  the solvent tendency of  $S$ , a quantity presumed to be entirely independent of the concentration  $s$  of  $S$  in the solution: any attempt at alteration of  $s$  by dilution will be fruitless. The physical explanation of this is, that the boundary surface between  $S$  and the solution does not in the least degree change its composition when the molecules, in order to maintain equilibrium, during the gradual dilution, flow out from  $S$  into the solution.  $P_s$  is a thermodynamic quantity of dimension as a concentration, and if it is desired to give it kinetic

content it can be expressed thus:  $P_s$  is proportional to the probability that a molecule  $S$ , under the influence of the thermic movement, passes through the field of force which exists in the zone between  $S$  and the solution, but also contains the proportionality factor with which the activity of the molecules must be multiplied to give the probability of a molecule passing from without through one or another boundary surface into this field of force.

If we now imagine  $S$  as very sparingly soluble, and another substance  $R$  added to the solution, then the molecules of  $R$  will be "adsorbed" at the boundary surface between  $S$  and the solution. Let us then imagine the adsorption as positive; a molecule  $R$  with the kinetic energy  $0$  placed in the field of force at the boundary zone will move towards  $S$ , i. e. an accumulation of  $R$  will take place at the boundary surface.

The state of equilibrium thus arrived at differs from that previously mentioned in the fact that the state of the boundary surface varies with the concentration  $c_i$  of  $R$  in the solution, and consequently, at a given temperature, all concentrations of  $R$  are possible, as long as they lie within the concentration of saturation for  $R$  at the temperature in question.

The task of the adsorption chemist is now to investigate how the quantity of matter accumulated in a state of equilibrium at the boundary surface varies with the concentration of the solution, and we here get different adsorption isotherms according to the theories on which our calculation are based. Several interesting endeavours have been made to establish generally valid formulæ (Polanyi, Langmuir, Freundlich); nevertheless, it must be admitted that we are far from mastering this difficult field as yet.

In the foregoing, it was presumed that the molecules of  $R$  did not carry any electric charge. A common feature of all forces acting in the adsorption zone on such molecules ("chemical valency forces", van d. Waal's cohesion forces, repulsive forces) is the fact that they act at very short distances and become infinitesimal on reaching a distance of only 1 or two molecule diameters from the boundary surface, which is related to the fact that they are inversely proportional to a very high power of the distance (see for instance Debye<sup>1</sup>) and Linderstrøm-

<sup>1</sup>) Physik. Zeitschr. **21**, 178 (1920).

Lang<sup>1)</sup>). We can therefore, by laying a surface A in the solution close up to the boundary surface, divide the molecules of R into two kinds, adsorbed and non-adsorbed, which further makes it possible to speak of a certain tendency  $P_i$  on the part of the molecules to pass A on the way out. And further, we have as before  $a_i = P_i$  at equilibrium.

Presuming now that the molecules of R carry electric charges, and that it is, say, the positive ions of R which are adsorbed, then the adsorption layer will be positively charged as relative to the solution, and the electric field thus arising on the boundary surface will now, owing to the low power,  $\frac{1}{2}$ , of the distance, contained in Coulomb's Law, extend out into the solution far beyond the field of the molecular forces, and an ion entering from without will, on reaching A, already have traversed the greater part of the electric field. If the solution be conducted to the ground, and supposing the potential in A to be X, then the energy required for the ion to traverse the field will be  $\varepsilon \cdot X \cdot i$  where  $i$  is the valency of  $R^+$ . If the concentration of  $R^+$  in the solution be called  $c_i$  then the concentration in a volume element situated in A will be:

$$c_i \cdot e^{-\frac{Xi\varepsilon}{kT}} \text{ and the activity therefore } a_i \cdot e^{-\frac{Xi\varepsilon}{kT}}.$$

For the ion equilibrium therefore, we have

$$P_i = a_i \cdot e^{-\frac{Xi\varepsilon}{kT}}. \quad (16)$$

If we now apply the Helmholtz theory for double layers  $X = \frac{4\pi\sigma a'}{D}$ , where  $a'$  is the distance between the double layers, and  $\sigma$  the electric surface density, then the quantity  $\sigma_i$  of ions adsorbed per cm will be expressed by:  $\varepsilon\sigma_i = \sigma$ .

$$\sigma_i = -\frac{DkT}{4\pi a' \cdot i \cdot \varepsilon^2} \ln \frac{P_i}{a_i}. \quad (17)$$

We will now use the term simple ion adsorption for cases where we can, within certain limits of concentration of the ion adsorbed, reckon  $P_i$  as constant. A typical instance of this is afforded by the metal electrodes. Another example may also be

<sup>1)</sup> »On The Salting out Effect«, Comptes rendus du Lab. Carlsberg **15**, 4 (1923).



mentioned, as being of importance for what follows, to wit, the adsorption of hydrogen ions on the boundary surface between a solid amino-acid ( $\text{NH}_4^+ - \text{R} - \text{COO}^-$  or  $\text{NH}_2 - \text{R} - \text{COOH}$  see Bjerrum<sup>1)</sup>) and water, where the affinity in binding of the hydrogen ion at  $\text{COO}^-$  is exactly the same as the affinity in binding to the  $\text{NH}_2$ -group. For with any such amino-acid, providing it exists at all, the probability  $P_i$  will be independent of  $a_{\text{H}^+}$  as long as the quantity of hydrogen ions adsorbed is small in comparison with the number of amino-acid molecules in the boundary layer, i. e., when the solution approaches the isoelectric reaction of the amino-acid. This, however, is only valid as a rough approximation.

To appreciate the alteration of  $P_i$  with the concentration  $c_i$  it is necessary to determine what forces act in the inner layers of the boundary zone. There may be both "chemical valency forces" and ordinary molecular forces of attraction. The chemical valency forces must be characterised, in contradistinction to the ordinary molecular forces of attraction, as requiring a definite orientation of the molecules or atoms attracting one another, while an extremely slight dislocation of these relative to one another occasions a complete rupture of the link which unites them. This process of dissociation is generally followed by a great loss of kinetic energy of the molecules. In comparison with these forces, the molecular attraction, which in many respects plays a predominant part in adsorption, acts here at somewhat greater distances, though still very short, and further, orientation is not necessary here, and appears, where it occurs at all, as a mean orientation of the moving molecules. Moreover, the potential energy of these forces is as a rule small against that of the chemical forces. The transition from one kind of force to the other is hardly very sharp. As I have pointed out in a previous work (l. c.) the dislocations effected by the fields of force of two mutually reacting molecules on one another's electron systems might very well be supposed to occasion mutual attraction, even though the reaction were, for some reason or other, prevented from reaching completion.

In view of what is to follow, we will now choose a chemical, and, by the way, purely statistical, explanation of the al-

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<sup>1)</sup> Zeitschrift f. phys. Chem. **104**. 147 (1923).

teration in  $P_i$  with  $c_i$ , using a method entirely in accordance with that employed by Langmuir<sup>1)</sup> in formulating his adsorption expressions. It is possible to arrive at similar results by other means, to wit, by supposing the existence of molecular forces, but we can hardly inter into this question here. By way of example, we will take the above mentioned amino-acid at isoelectric reaction. At this concentration of hydrogen ions we should have, for instance,  $k$  places per  $\text{cm}^2$  where a hydrogen ion could settle down, and if we assume, after Bjerrum (l. c.) that the amino-acid is of "Zwitterion"-structure, it means that these places are negatively charged carboxyl groups. There are also  $k$  places from where a hydrogen ion can pass out, (viz.  $k$  positively charged substituted ammonium groups). The tendency  $P_i$ , which we have already mentioned, we can at this isoelectric reaction call  $P'_i$ . If we increase the concentration of hydrogen ions in the solution, the hydrogen ion answering to  $R$  with the term we used before, then some hydrogen ions will settle on the "open" spots, let us say  $\sigma_i$  per cm.  $P'_i$  will now, purely statistically, alter with the proportion  $\frac{k + \sigma_i}{k - \sigma_i}$ , and the new tendency will thus become  $P'_i \cdot \frac{k + \sigma_i}{k - \sigma_i}$ .

Preassuming  $\sigma_i$  to be small in comparison with  $k$ , this expression can be re-written  $P'_i \cdot e^{\frac{2 \sigma_i}{k}}$  and on inserting this value in (17) in place of  $P_i$ , we get

$$\sigma_i = - \frac{1}{4 \pi a' \cdot i \cdot \epsilon^2 / DkT + 2/k} \cdot \ln \frac{P'_i}{a_i}, \quad (17a)$$

reverting to the terms previously employed.

In this equation, both the alteration in the electric field at the boundary surface and the alteration in the state of the inner layer of the boundary — with the concentration of  $R^+$  — have been taken into consideration, (by way of example,  $H^+$  has been chosen). (17 a) thus represents an ordinary ion adsorption isotherm.

<sup>1</sup> See Freundlich: Kapillarchemie. Leipzig 1922. Pag. 175.

### C. Mean Valency and Law of Mass Action.

#### 1. *The term Mean Valency at constant ion concentration.*

We imagine the particles of a given substance S dissolved to the concentration  $c_k$  in water. By this we understand, that each litre contains  $N \cdot c_k$  particles ( $N = 6,06 \cdot 10^{23}$ ). We will further imagine the particles of S as capable of catching or giving off hydrogen ions, so that they would, by alteration in their concentration of hydrogen ions, be able to alter their valency.

We will now examine the alteration occasioned in the concentration of hydrogen ions by the addition of a certain quantity of a base or acid; in other words, we propose to investigate the capacity of S to combine with acids or bases. In order to simplify the matter, we will add to the solution a quantity of some salt, with so high a concentration  $c_E$  that both  $c_k$  and the concentrations of the added acid or base will be slight in comparison, though  $c_E$  must not be so high as to invalidate the basis for Debye's theory in Section A. The addition of this salt renders the concentration of hydrogen ions proportional to their activity.

To a litre of the solution we now add  $dy$  equivalents of a strong acid or base,  $dy$  being reckoned as positive, when acid is added, negative when adding a base. This occasions, in the first place, of course, an alteration of the activity of hydrogen ions  $a_H$ ,  $da_H$ , proportional to the alteration in concentration of hydrogen ions  $c_H$ ,  $dc_H$  as  $da_H = f_H dc_H$  where  $f_H$  is the activity coefficient for the hydrogen ion at the salt concentration noted. And further, the activity of the hydroxyl ions will be altered, as

$$a_H \cdot a_{OH} = k_W \text{ (} k_W \text{ dissociation constant of the water)}$$

$$\text{or} \quad da_{OH} = -\frac{k_W}{a_H^2} da_H$$

$$\text{or} \quad dc_{OH} = -\frac{k_W}{f^2 \cdot c_H^2} \cdot dc_H, \text{ setting } f_H = f_{OH} = f.$$

If now the particles be so great that we can imagine them in phase equilibrium with the solution, we can give them all the same valency  $n$ , determined by (17) or (17a),  $n$  being the total charge of the particle divided by the electric elementary quantity. As the variation in the valency is positive, when acid: hydrogen ions, are added, we must, for the relation between variation in

concentration of hydrogen ions, valency and acid concentration, have the following equation:

$$dy = dc_H - dc_{OH} + c_k \cdot dn. \quad (18)$$

It cannot be sufficiently emphasised, however, that there will ordinarily be no real physical justification for treating the particles in this manner. It would be far more in accordance with simple views to suppose that the particles have not all the same charge, but that the charge varies discontinuously from one particle to another with one elementary charge. If  $c_r$  be the concentration of particles of the valency  $r$ , then we can define a mean valency  $n_m$

$$n_m = \frac{\sum r \cdot c_r}{\sum c_r}, \quad (19)$$

where the summation is to comprise all particles, and all possible valencies,  $\sum c_r = c_k$ . Thus defined,  $n_m$  enters precisely as  $n$  in (18), and by (18) then, we can determine the variation of the mean valency with addition of acid or base and concentration of hydrogen ions.

Transforming (18) we obtain:

$$\frac{dy}{da_H} = \frac{1}{f} \left( 1 + \frac{k_w}{a_H^2} \right) + c_k \frac{dn_m}{da_H}. \quad (20)$$

$a_H$  and  $\frac{da_H}{dy}$  being determined by potential measurement, we can then, provided  $c_k$  be known (i. e. the particle magnitude of  $S$  in the solution and total weight of same in a litre) find the variation of  $n_m$  with the activity of hydrogen ions.

### 2) Calculation of the Mean Valency.

And now, how does  $n_m$  vary with  $a_H$ ? This depends naturally on the suppositions we entertain as to the qualities of the molecules or particles. Let us assume the following simple ideas as to their structure. The particles are spherical, with radius  $a$ , the dielectricity constant  $D$ . The charge is placed in the centre of the sphere, and when a hydrogen ion settles on or moves away from the sphere, it passes on its charge to the centre of the sphere, or draws it from the same, as the case may be. When a particle has the charge 0, then there will be at its surface  $q$  places at which a hydrogen ion can be bound, and  $q$

places from which it can be dissociated, and the probability of binding and dissociation is the same for all  $2q$  places. If the charge for instance be  $+3$ , there are  $q-3$  "open" places and  $q+3$  "closed" places, and the probability of binding is now, if we disregard the influence of the charge,  $q-3/q$  times less, the probability of dissociation  $q+3/q$  times greater than before.

Now the particles in our solution are in thermodynamic equilibrium, and for two particles with the valency  $r = -z$  and  $r + 1 = -z + 1$ , where  $z$  is a positive whole number, the following equation of mass action will apply:

$$a_{-z} \cdot a_H = k \cdot a_{-z+1}, \quad (21)$$

and the question then is to determine how  $k$  depends on  $z$ .

Bjerrum<sup>1)</sup> has shown, that with dibasic acids, e. g. adipinic acid, it is possible, from the relation between the two dissociation constants, to calculate a value for the distance between the two carboxyl groups which will be correct as regards its order of magnitude — when taking into consideration the charge of the monovalent ion and the alteration in statistical probability of dissociation and binding of the hydrogen ion with the number of "places" as above mentioned. Generally speaking — with infinitely diluted ionic solutions, that is to say for  $a = c$  — the calculation for the two above mentioned ions will appear as follows:

At the surface of the ion with the charge  $-z$ , the potential according to (11) is

$$\psi = -\frac{z \cdot \epsilon}{D \cdot a}, \quad \text{for } c = 0 \text{ being } 0. \quad \text{If the concentration of}$$

hydrogen ions in the solution be  $c_H$ , then, according to Boltzmann's theorem it will be

$$c_H \cdot e^{-\frac{q\epsilon}{kT}} = c_H \cdot e^{\frac{z \cdot \epsilon^2}{DaKT}} \quad \text{at the surface, or more precisely ex-}$$

pressed, the probability of finding a hydrogen ion in a volume element situated at the surface of the ion is the volume element multiplied by  $c_H \cdot e^{\frac{z \cdot \epsilon^2}{DaKT}}$

The number,  $X$ , of ions of the valency  $-z + 1$  dissociated

<sup>1)</sup> Zeitschrift f. phys. Chem. **106**, 219 (1923).

per unit of time in a hydrogen ion and an ion of the valency  $-z$ , is now proportional with  $c_{-z+1}$  in the first place, and further, with the number of hydrogen ions which can be dissociated, i. e. from the foregoing, with  $q - z + 1$ . We thus obtain, for  $X$ ,

$$X = k' (q - z + 1) \cdot c_{-z+1}.$$

The number  $Y$  of ions of the valency  $-z + 1$  formed per unit of time by hydrogen ions and ions of the valency  $-z$  is however, in the same way

$$Y = k'' (q + z) \cdot e^{\frac{z \cdot \varepsilon^2}{DakT}} c_H \cdot c_{-z},$$

and when there is equilibrium, i. e. when  $X = Y$ , we have in other words the following:

$$c_{-z} \cdot c_H = k_o \cdot \frac{q - z + 1}{q + z} \cdot c^{-\frac{z \cdot \varepsilon^2}{DakT}} \cdot c_{-z+1}, \quad (22)$$

where  $\frac{k'}{k''} = k_o$ .

In an ion solution where the concentration is not infinitesimal, but answers to what we have imagined as to the solution of  $S$ , we should now have  $k$  in the mass action expression (21) identical with the constant in (22), that is:

$$a_{-z} \cdot a_H = k_o \cdot \frac{q - z + 1}{q + z} \cdot c^{-\frac{z \cdot \varepsilon^2}{DakT}} \cdot a_{-z+1}. \quad (23)$$

This equation we will use for the calculation of the mean valency. In so doing, it will be more practical to divide all particles into the positive and the negative, which gives us the following equations:

Valency $r$ negative.	Valency $r$ positive.
$a_{-z} \cdot a_H = k_{-z} \cdot a_{-z+1}$	$a_{z-1} \cdot a_H = k_{z-1} a_z$
$a_{-z+1} \cdot a_H = k_{-z+1} \cdot a_{-z+2}$	$a_{z-2} \cdot a_H = k_{z-2} \cdot a_{z-1}$
.....	.....
.....	.....
$a_{-1} \cdot a_H = k_{-1} \cdot a_o$	$a_o \cdot a_H = k_o \cdot a_1$
$a_{-z} \cdot a_H^z = (k_{-1} \cdot k_{-2} \dots) a_o$	$a_z \cdot a_H^{-z} (k_o \cdot k_1 \dots) = a_o$

(24)

The two equations (24) which are formed from the others by multiplication will, on introducing (23) be transformed into:

$$a_{-z} = \frac{(q-1+1)(q-2+1)\dots(q-z+1)}{(q+1)(q+2)\dots(q+z)} \cdot \left(\frac{k_0}{a_H}\right)^z \cdot e^{-yz(z+1)} \cdot a_0$$

$$a_z = \frac{(q-1+1)(q-2+1)\dots(q-z+1)}{(q+1)(q+2)\dots(q+z)} \cdot \left(\frac{k_0}{a_H}\right)^{-z} \cdot e^{-yz(z-1)} \cdot a_0,$$

where  $y = \varepsilon^2/2DkT\alpha$ . Inserting  $r = -z$  or  $r = +z$  respectively, these two equations pass over into the following single equation:

$$a_r = \frac{(q-1+1)(q-2+1)\dots(q-|r|+1)}{(q+1)(q+2)\dots(q+|r|)} \cdot \left(\frac{k_0}{a_H}\right)^{-r} \cdot e^{-yr(r-1)} \cdot a_0, \quad (25)$$

where  $|r|$  is the numerical value of  $r$ . In order to find  $c_r$  it will now be necessary to insert  $a_r = c_r \cdot f_r$  where  $f_r$  is determined by (14)

$$\ln f_r = - \frac{x}{2DkT} \cdot \varepsilon^2 r^2 \frac{1}{1+xa}.$$

$$\text{If we now set } \ln P_0 = \ln k_0 - \frac{\varepsilon^2}{2DakT} = \ln k_0 - y \quad (26)$$

and

$$w = \frac{1}{2} \left( \frac{\varepsilon^2}{DakT} - \frac{x\varepsilon^2}{DkT(1+xa)} \right) = \frac{\varepsilon^2}{2DakT(1+xa)} \quad (27)$$

(25) will be simply transformed into

$$c_r = \frac{(q-1+1)(q-2+1)\dots(q-|r|+1)}{(q+1)(q+2)\dots(q+|r|)} \cdot e^{-wr^2 - r \ln P_0 / a_H} \cdot a_0 \quad (28)$$

Let us now imagine ourselves within the limits of hydrogen ion concentration where the ions whose numerical valency is not small in comparison with  $q$  are of so slight a concentration that they contribute nothing to the mean valency. This will render (28) extremely simple in form, as the first term on the right side disappears:

$$c_r = e^{-wr^2 - r \ln P_0 / a_H} \cdot a_0,$$

and the mean valency, which is determined by (19), then becomes:

$$n_m = \frac{\sum e^{-wr^2 - r \ln P_0 / a_H} \cdot r}{\sum e^{-wr^2 - r \ln P_0 / a_H}}, \quad (29)$$

$a_0$  being cancelled out. The summation must comprise all particles. In order to utilise this expression we will first ascertain for what value the term

$$e^{-wr^2 - r \ln P_0 / a_H} \text{ has its maximum.}$$

This will evidently be the case when

$$r = r_m = -\frac{1}{2w} \ln \frac{P_0}{a_H}, \quad (30)$$

which can be confirmed by differentiation, and by inserting this value for  $\ln \frac{P_0}{a_H}$  in (29) and dividing numerator and denominator by  $e^{wr_m^2}$  we therefore obtain:

$$n_m = \frac{\sum e^{-w(r-r_m)^2} \cdot r}{\sum e^{-w(r-r_m)^2}}. \quad (31)$$

The difference between  $n_m$  and  $r_m$  we may call  $A$ , whereby  $A$  will be determined by

$$A = \frac{\sum e^{-w(r-r_m)^2} (r-r_m)}{\sum e^{-w(r-r_m)^2}}. \quad (32)$$

This function  $A$  is of a complicated periodical character. It is 0 for

$$r_m = \frac{i}{2} \quad (i = 1, 2, \dots, -1, -2, \dots),$$

when  $r$  passes through all whole values from  $-\infty$  or  $+\infty$  that is to say, that  $n_m$  and  $r_m$  are of equal magnitude for these values of  $r_m$ . It is interesting, however, that  $A$  approaches with ever increasing accuracy to the value 0 for any value of  $r_m$  when  $w$  approaches 0. For in such case we can in (22) substitute for the summation sign the corresponding integral:

$$A = \frac{\int_{-\infty}^{+\infty} e^{-w_0(r-r_m)^2} (r-r_m) d(r-r_m)}{\int_{-\infty}^{+\infty} e^{-w_0(r-r_m)^2} d(r-r_m)}, \quad (33)$$

where the numerator is 0 for any value of  $r_m$  while the denominator remains finite.

What now does this mean, that  $w$  approaches 0? It means that we allow the radius of the particles to increase (see pag. 15). If we imagine the charge of the particles evenly distributed about



the surface instead of at the centre, then  $r_m = 4\pi a^2 \sigma_m$  and consequently, according to (30)  $\sigma_m$  is equal to

$$\sigma_m = -\frac{1}{8\pi a^2 w} \ln \frac{P_0}{a_H}.$$

Inserting the value of  $w$  from (27) into this equation, and allowing  $a$  to increase, we obtain:

$$\lim \frac{r_m}{4\pi a^2} = \lim \frac{n_m}{4\pi a^2} = \lim \sigma_m = -\lim \frac{1}{4\pi a^2} \cdot \frac{DakT(1+xa)}{\epsilon^2} \cdot \ln \frac{P_0}{a_H} =$$

$$-\frac{DkTx}{4\pi \epsilon^2} \ln \frac{P_1}{a_H} \dots \dots \dots (34)$$

an expression identical with (17) when  $\frac{1}{x}$  (34) is set in place of  $a'$  and  $i$  as equal to 1 for the hydrogen ion.

The agreement between (17) and (34) gives, it seems to me, a very clear view of the continuity which must exist between the adsorption theory on the one hand and the law of mass action on the other, a continuity which, as mentioned in the introduction, was to be expected.

Table 1.  
Relation between  $n_m$  and  $r_m$ .

$w = 23.02$ $a = \text{ca. } 10^{-9} \text{ cm}$		$w = 2.422$ $a = 1.4 \cdot 10^{-8}$		$w = 0.2669$ $a = 10^{-7}$	
$r_m$	$n_m$	$r_m$	$n_m$	$r_m$	$n_m$
—1.0	—1.000	—0.9504	—0.9634	—0.8629	—0.8629
—0.8	—1.000	—0.7624	—0.8063	—0.5685	—0.5685
—0.6	—0.990	—0.7128	—0.7595	—0.4315	—0.4315
—0.55	—0.909	—0.5248	—0.5320	—0.1573	—0.1580
—0.52	—0.666	—0.4752	—0.4680	—	—
—0.50	—0.500	—0.2872	—0.2405	—	—
—0.48	—0.334	—0.2376	—0.1937	—	—
—0.45	—0.091	—0.0496	—0.0366	—	—
—0.40	—0.010	—	—	—	—
—0.2	—0.000	—	—	—	—
—0.0	—0.000	—	—	—	—

The suppositions regarding structure of the molecules and the affinity to the hydrogen ions which we have here entertained are naturally very simple, and only very roughly in accordance

with reality, but on the other hand, they are no worse than those we are often constrained to employ, e. g. in gas theoretical calculations. The importance of these suppositions will be discussed later on. I must only point out here that they must of

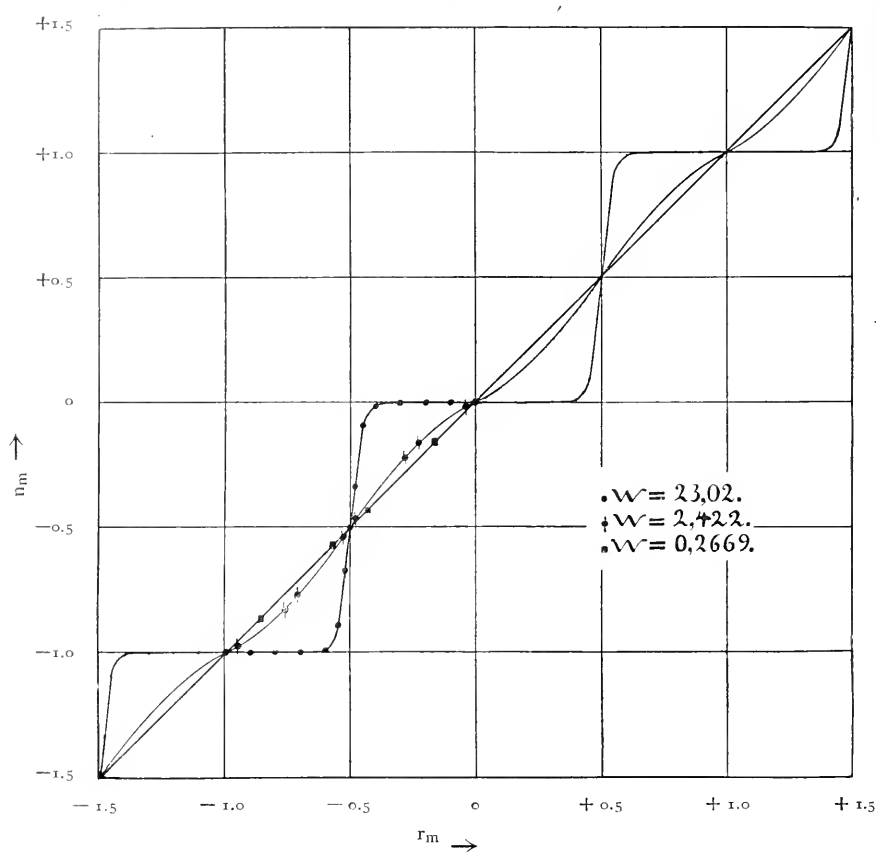


Fig. 1.

course not be taken to contain either more or less than the suppositions on which formula (17) is based (see pag. 8).

If we do not desire to go to the limit as in (34) where  $a$  increases to infinity, but wish to examine the agreement between  $n_m$  and  $r_m$  for a finite  $a$ , it is interesting to note that the expression

$$n_m = r_m = - \frac{DakT (1 + xa)}{\varepsilon^2} \cdot \ln \frac{P_0}{a_H} \quad (35)$$

is an excellent approximation for (29) when  $a$  is  $10^{-7}$  or more, as at this value of  $a$ , with the accuracy which the calculation per-

mits, it is altogether impossible to demonstrate any difference between  $n_m$  and  $r_m$ . This is best seen from Table 1 and Figure 1. The calculation has here been made with three different sizes of particles, and correspondingly varying values for  $w$ , the concentration of ions being taken as  $=0.01$  n KCl.

In the foregoing, the factor

$$F = \frac{(q-1+1)(q-2+1) \dots (q-lr+1)}{(q+1)(q+2) \dots (q+l)}.$$

has been disregarded. By a simple approximation, however, it can be brought into (29) and (30) without altering their form. According to the definition of the base of the natural logarithms  $e$ ,  $F$  can, when  $r$  is not great in proportion to  $q$ , be written:

$e^{-r^2/q}$ , (29) thus being transformed into:

$$n_m = \frac{\sum e^{-(w+1/q)r^2 - r \ln P_0/a_H} \cdot r}{\sum e^{-(w+1/q)r^2 - r \ln P_0/a_H}} \quad (36)$$

and (30) to

$$r_m = - \frac{1}{2(w+1/q)} \ln \frac{P_0}{a_H}. \quad (37)$$

If, in (37) we allow  $w$  to go towards 0, we must bear in mind that as the particle radius increases, so does  $q$ , the number of groups in the particle, in all probability also. As  $q$  is the number of places on the surface of the ions, it is reasonable to set  $q = k \cdot 4\pi a^2$ .  $1/q$  thus comes to contain  $a^2$  in the denominator just as  $w$ , and when we go to the limit, so noted on p. 17 then the expression for  $\sigma_m$  answering to the adsorption expression will contain  $k$ , as

$$\lim \sigma_m = - \lim \frac{1}{8\pi a^2 (\epsilon^2/2DkT(I + xa) + 1/k \cdot 4\pi a^2)} \cdot \ln \frac{P_0}{a_H} = - \frac{1}{4\pi \epsilon^2/DkTx + 2/k} \cdot \ln \frac{P_0}{a_H}, \quad (37a)$$

an expression which is identical with (17a) when  $i$  is taken as equal to 1 and  $x = \frac{1}{a}$ .

The degree of approximation with which  $n_m = r_m$  (37) reproduces

$$n_m = \frac{\sum F \cdot e^{-wr^2 - r \ln P_0/a_H} \cdot r}{\sum F \cdot e^{-wr^2 - r \ln P_0/a_H}}, \quad (38)$$

will be seen from Table 2.

Table 2.

$w = 0,2669$ ,  $a = 10^{-7}$ .

$p_{aH}$	$r_m$	$n_m$	$n_m(38)$	$n_m(38)$	$r_m(37)$	$r_m(37)$
			$q=5$	$q=10$	$q=5$	$q=10$
4.0	4.3146	4.315	—	—	2.466	3.139
4.2	3.4517	3.452	1.973	2.519	1.973	2.511
4.5	2.1573	2.158	—	—	1.233	1.570
4.8	0.8629	0.863	0.472	0.634	0.493	0.627
4.9	0.4315	0.432	—	—	0.247	0.314
5.0	0	0	0	0	0	0

In these calculations, S is imagined as situate in a KCl solution of the concentration 0.01 N as in Table 1. Thus  $x = 3.2850 \cdot 10^6$  and by inserting this value and  $a = 10^{-7}$  in (27)  $w$  is calculated to 0.2669. We have further, through the foregoing, given S the character of an ampholyte with acid groups — tendency to give off hydrogen ions — and base groups — tendency to take up hydrogen ions (cf. J. N. Brønsted<sup>1)</sup>) and the isoelectric reaction of this ampholyte is according to (30) or (37), determined by  $a_H = P_0$ . In Table 2,  $P_0$  is taken as equal to  $10^{-5}$  (the isoelectric reaction for many proteins).  $p_{aH} = -\log a_H$ .

Figure 2 also shows graphically, in curves 1, the dependence of  $r_m$  on  $p_{aH}$ . The fully drawn curve is that for  $r_m$ , the dotted curve that for  $r_m(37)$  ( $q=10$ ) and the stippled curve that for  $r_m(37)$  ( $q=5$ ).

The curve marked II is the curve for  $r_m$  when  $w$  is equal to 2.422, i. e.  $x = 3.2850 \cdot 10^6$ ,  $a = 1.4 \cdot 10^{-8}$ . The dotted wave line is the corresponding curve for  $n_m$ .

The actual contents of these observations may be summed up as follows:

With a dibasic acid, such as for instance adipinic acid, there is a definite proportion between the dissociation constants of the two stages  $k_1$  and  $k_2$ :

$$\begin{aligned} c_{S-} \cdot c_H &= k_1 \cdot c_S \\ c_{S--} \cdot c_H &= k_2 \cdot c_{S-}. \end{aligned}$$

If the two carboxyl groups are situated far apart and are

<sup>1)</sup> Rec. de Trav. Chim. d. Pay-Bas. **62**, 718 (1923).

perfectly symmetrical, as in adipinic acid, there are two factors which determine this proportion.

In the first place,  $k_1$  is greater than  $k_2$  because the hydrogen ion, owing to the electrostatic attraction, is more likely to settle on the doubly charged ion than on that with a single charge.

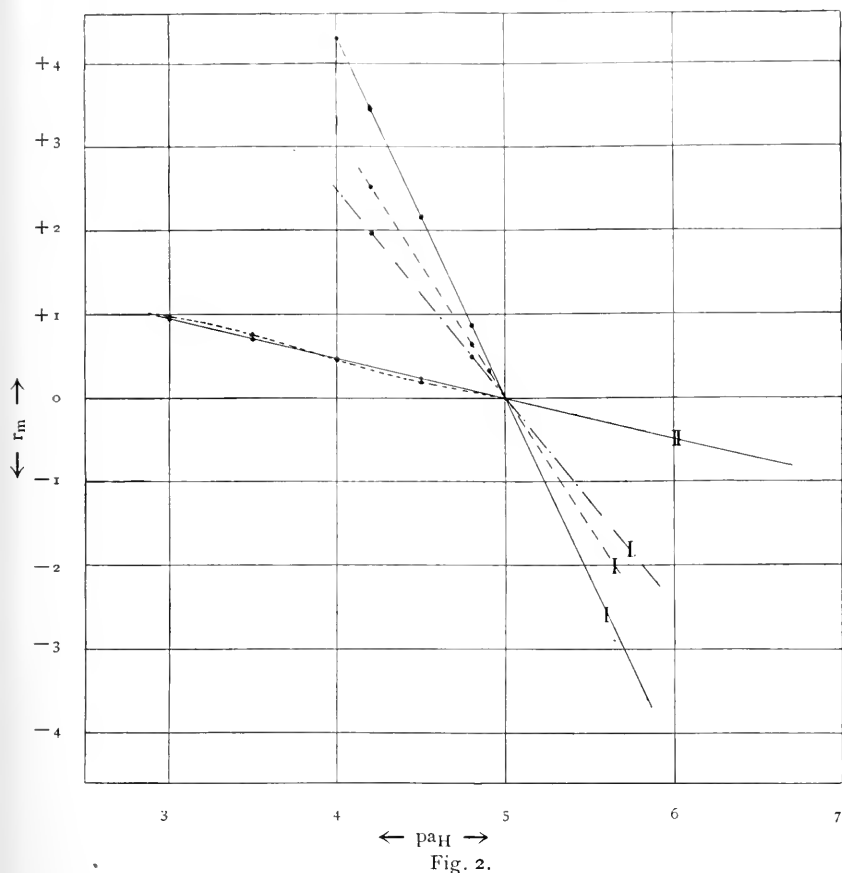


Fig. 2.

Secondly, the probability that a hydrogen ion should settle on a molecule with two "open" places, the doubly charged ion, is as a matter of mere statistics, twice as great as the probability of its settling on a molecule with one open place, (the singly charged ion). In the same way, the probability that a hydrogen ion should be dissociated from a molecule with two "closed" places, the uncharged molecule, is twice as great as in the case of the singly charged ion.  $k_1$  will thus be 4 times as great as  $k_2$ .

From Bjerrum's simple expression  $k_1 = 4 \cdot e^{e^2/DkTa} \cdot k_2$ , where  $a$  is the distance between the carboxyl groups, it will be seen that the greater  $a$  is, the more will the  $e$ -expression approach 1, and the nearer will the proportion of the two dissociation constants approach 4. If, as  $a$  increases, we let the number of open and closed places increase, the ratio approaches 1, as the statistical factor 4 alters with the number of places, and becomes 1 when this is very high. And as the number of places increases, so also an increase takes place in the number of dissociation stages, and since the dissociation constants lie close together, there will be several different types of ions with different valencies determining the mean valency. For instance, in the summation expression (29) when  $w = 23,02$ ,  $a = \text{ca. } 10^{-9}$  there will be at the outside two terms that signify, when  $w = 2,422$ ,  $a = 1,4 \cdot 10^{-8}$  4—6 such terms, and when  $w = 0,2669$ ,  $a = 10^{-7}$  10—12. It is the dissociation curves of these separate stages which, when the dissociation constants with increasing particle radius, and decreasing  $w$  approach one another more, overlap in a highly complicated function, which, however, as  $a$  increases, can be reproduced with ever increasing accuracy by the equation (37):

$$n_m = r_m = - \frac{1}{2(w + 1/q)} \cdot \ln \frac{P_0}{a_H}$$

which is a simple adsorption formula, linear with regard to  $\text{p}a_H$ .

On glancing at (20), it will be seen that if  $n_m$  can be calculated from (37) it is possible, by acid binding or base binding

experiments, to determine  $\frac{c_k}{2(w + \frac{1}{q})}$  and if we here again, as

with the formulation of (35) disregard the term  $1/q$ , as small in comparison with  $w$ , then  $c_k/2w$  can be determined. As  $c_k$  can

be written  $\frac{g \cdot d}{N \cdot \frac{4}{3} \pi a^3}$  where both  $g$ , the number of grams of S

per litre, and  $d$ , the specific gravity of the particles, can be measured, and  $w$  can be calculated for the electrolyte concentration employed,  $c_k/2w$  has only one unknown quantity,  $a$ , which can thus be determined in this manner by acid or base binding experiments.

We will now finally see how these views hold good for protein substances.

## D. Ionisation of Proteins.

### 1) *General observations.*

It was pointed out in the introduction that great care should be taken in applying ordinary adsorption theories to the problem of proteins, partly because the protein particles are too small for the ordinary boundary surface mode of observation, and also because the ordinary adsorption theory often gives a very inadequate and too general view of difficult problems and thus constitutes a positive danger to the experimental work; that is to say, it tends to hinder accurate study of the individual qualities of the substances concerned.

The importance of thorough experimental work has already been pointed out by S. P. L. Sørensen "Studies on Proteins" (l. c.) and I am convinced that the views on which that work is based are correct in the main, though the development of the theory of strong electrolytes was, in 1917, not very far advanced. As regard the problem of ionisation, the simple application of the principle of mass action given in "Studies on Proteins" could not lead to final results, a fact of which Sørensen was also well aware; but when it is maintained, from the point of view of adsorption theory, that the law of mass action is altogether inapplicable to a colloid substance such as for instance egg albumin, this must, in consideration of the observations in the foregoing section, be said to be erroneous. The fact is simply this, that in egg albumin, of which the substance S (see pp. 8 and 12) may be said to be an ideal representation, we have a substance with many acid and base groups, whose dissociation constants do not lie far apart, and whose dissociation curves therefore partly overlap and efface each other. This idea is not new, and has often been impressed upon me by Sørensen himself; it has simply been my task here to pursue the idea, by simple suppositions, to its logical conclusion, thereby revealing its agreement with a simple adsorption theory. This agreement might perhaps argue in favour of the application of the adsorption expression, which is far simpler than the corresponding mass action expression, but it must emphatically be pointed out that this cannot be done in a general way, since the particular suppositions we have entertained (that the charge is situated in the centre; that the affinity of the hydrogen ion to all  $2q$  places is

the same), can only with rough approximation apply to proteins in general. And that for instance the presence of a strong acid group, e. g. a phosphoric acid group, in the protein molecule can dislocate the picture to an essential degree, at any rate within definite limits of hydrogen ion concentration, and that suppositions as to orientation of the charges in the outer layer of the particles render the foregoing observations extremely difficult.

If, despite these scruples, I endeavour to carry out the calculation on the basis of some experiments by Sørensen on the acid-binding power of egg albumin, it is because the character of this substance, neither particularly strongly acid nor strongly basic, might possibly warrant the application of our formulæ, and also because it is always interesting to ascertain how far one can get with an expression for mean valency such as (37).

That our suppositions as to the nature of the substance s roughly coincide with the view we can form of the qualities of protein substances would seem beyond doubt. In an aqueous solution, we must imagine the molecules of these substances as composed of numerous molecules of different kinds, united by forces, the nature of which we still know very little about. As the molecules of which these complexes are formed are, at any rate, for a great part, amino-acids, there is no slight possibility for the existence of several base and acid groups in the outer surface of the molecule. Taking into consideration Bjerrum's supposition as to "Zwitter" ions, which enables us to imagine a particle of slight valency, yet with a certain number of electric charges distributed throughout the particle, the supposition as to the placing of the charge in the centre becomes more easily understandable (according to the laws of electrostatics, a charged particle acts uniformly whether the charge be in the centre or evenly distributed throughout its surface). But we cannot get beyond a rough approximation.

## 2. Comparison with Experimental Results.

By integrating equation (20) we get

$$y = \frac{1}{f} \left( a_H - \frac{k_w}{a_H} \right) + c_k \cdot n_m + i, \quad (39)$$



where  $i$  is determined by the initial state. If we then choose  $y = 0$  for  $n_m = 0$  i. e. at isoelectric reaction, then

$$i = - (a_H^0 - \frac{k_w}{a_H^0}) \cdot \frac{1}{f},$$

where  $a_H^0$  is the activity of hydrogen ions at isoelectric reaction. (39) will then be transformed to

$$c_k \cdot n_m = \frac{1}{f} ((a_H^0 - a_H) - (\frac{k_w}{a_H^0} - \frac{k_w}{a_H})) + y. \quad (40)$$

This equation is now suited to the direct insertion of the values found by Sorensen. A specially suitable series of experiments is shown in "Studies on Proteins", in the Table p. 126—127, the two last columns, where the concentration of ammonium sulphate is only 0.06 n. In Table 3, this series of experiments is reproduced, together with the figures necessary for our calculations.

Table 3.

a) $\alpha = 31.7$ $c(\text{NH}_4)_2\text{SO}_4 = 0.065.$						
$h \cdot 10^6$	$b = \frac{t-t^1}{\alpha}$	pH	p <sub>aH</sub>	$\alpha$	$t-t^1$	$\frac{c_k \cdot n_m}{(t-t^1) \cdot 10^{-5}}$
101.7	26.0	3.993	4.043	31.7	824	0.00824
87.24	23.4	4.059	4.109	—	742	742
70.86	20.6	4.149	4.199	—	653	653
57.50	17.5	4.240	4.290	—	555	555
45.08	13.9	4.346	4.396	—	441	441
31.49	9.3	4.502	4.552	—	295	295
20.83	3.5	4.681	4.731	—	111	111
15.23	0.0	4.817	4.867	—	0	0

b) $\alpha = 10.5$ $c(\text{NH}_4)_2\text{SO}_4 = 0.059.$						
$h \cdot 10^6$	$b = \frac{t-t^1}{\alpha}$	pH	p <sub>aH</sub>	$\alpha$	$t-t^1$	$\frac{c_k \cdot n_m}{(t-t^1) \cdot 10^{-5}}$
98.70	25.3	4.006	4.056	10.5	266	0.00266
85.44	22.9	4.068	4.118	—	240	240
70.85	20.5	4.150	4.200	—	215	215
56.71	17.5	4.249	4.296	—	184	184
45.93	13.8	4.338	4.388	—	145	145
33.57	9.4	4.474	4.524	—	99	0.00099
21.43	3.5	4.669	4.719	—	37	37
16.06	0.0	4.794	4.844	—	0	0

$h$  here indicates concentration of hydrogen ions, (calculated according to the classical dissociation theory),  $t$  is the quantity of acid added, noted as the number of cubic centimetres  $n/1000\text{H}_2\text{SO}_4$  added to 100 cc solution,  $t^1$  is the surplus of sulphuric acid present in 100 cm of the experimental liquid, a quantity entirely corresponding to the first term on the right side of (40) with opposite sign, as long as we take, for sulphuric acid

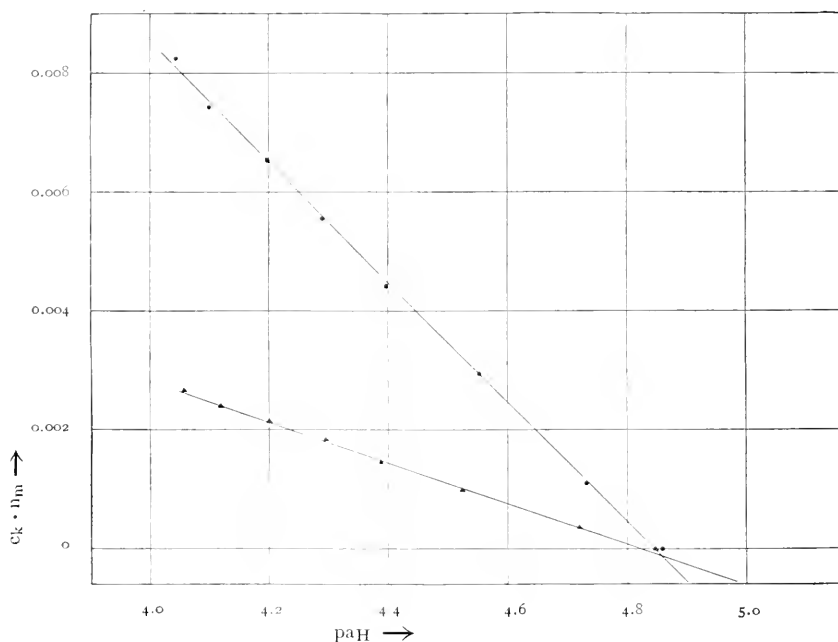


Fig. 3.

which is not completely dissociated,  $f$  as the factor by which the concentration of sulphuric acid (concentration of hydrogen ions + concentration of non-dissociated sulphuric acid) must be multiplied in order to give the activity of hydrogen ions in the solution.  $o$  is the no. of milligram equivalents of protein-nitrogen per 100 cc experimental liquid,  $p_H$  and  $p_aH$  have the usual significance. Multiplying  $t$ , which answers completely to  $y$  in (40), and  $t^1$  by  $10^{-5}$  we have expressed the concentration of acid as normalities in the solution in question, whereby  $c_k \cdot n_m$  according to (40) obtains the values shown in the last column. I have only included measurements made on the acid side of the isoelectric reaction, as the values round

and on the basic side of isoelectric reaction are probably more uncertain, owing to the incipient hydrolysis of the ammonium sulphate. Furthermore, there are too few determinations to afford any certain conclusions as to the alkaline course of the curve for  $c_k \cdot n_m$ .

In Fig. 3, the dependence between  $pa_H$  and  $c_k \cdot n_m$  is shown graphically.

The curves run, as will be seen, altogether in a straight line within the limits we have included. This rectilinear course agrees well with (37) when we remember that  $pa_H$  is equal to  $-\log a_H$ .

The radius of the particles of the protein a, can now be determined in two ways.

A. The first way is by going through the determination of osmotic pressure of protein solutions.

If the molecular weight of egg albumin be 35000,  $6.06 \cdot 10^{23}$  Avogadro's constant, 7.00 the factor by which the quantity of nitrogen in grams must be multiplied to give the weight of the protein (see Studies on Proteins, 364) 1.27 the specific gravity of the egg albumin particles, a their radius, we obtain

$$1) \quad c_k = \frac{0 \cdot 14.01 \cdot 7.00 \cdot 10^{-2}}{1.27 \cdot \frac{4}{3} \pi \cdot a^3 \cdot 6.06 \cdot 10^{23}} = 3.042 \cdot 10^{-25} \cdot \frac{0}{a^3}$$

$$2) \quad c_k = \frac{0 \cdot 14.01 \cdot 7.00 \cdot 10^{-2}}{35000} = 2.802 \cdot 10^{-5} \cdot 0.$$

These two values for  $c_k$  enable us to calculate the particle radius:

$$a = \sqrt[3]{\frac{3.042}{2.802} \cdot 10^{-20}} = 2.21 \cdot 10^{-7} \text{ cm,}$$

a value of a which is thus determined by osmotic measurements.

B. The value of a which we should, from the foregoing, be able to calculate from acid binding experiments, is obtained as follows:

If, in (37) we disregard the term  $1/q$ , this equation passes over into (35)

$$n_m = - \frac{DakT(1 + xa)}{\varepsilon^2} \ln \frac{P_0}{a_H} = K - \frac{DakT(1 + xa)}{0.4343 \varepsilon^2} pa_H$$

from which

$$\frac{dc_k \cdot n_m}{d pa_H} = c_k \cdot \frac{dn_m}{d pa_H} = -c_k \cdot \frac{DakT(1 + xa)}{0.4343 \varepsilon^2} =$$

$$= - \frac{3.042 \cdot 10^{-25}}{0.4343} \cdot 0 \cdot \frac{DkT(1 + xa)}{\varepsilon^2 a^2}$$

$x$ , is at the concentration  $0.06 \text{ n Am}_2\text{SO}_4$   $9.85 \cdot 10^6$  and inserting this value and the numerical values of  $D$ ,  $k$ ,  $T$ ,  $\varepsilon$ ,  $o$  and  $\frac{dc_k \cdot n_m}{d\mu_H}$  read from the straight lines in figure 3, we obtain:

$$a) \quad o = 31.7; \quad \frac{dc_k \cdot n_m}{d\mu_H} = -0.01009$$

$$a = 3.86 \cdot 10^{-7} \text{ cm.}$$

$$b) \quad o = 10.5; \quad \frac{dc_k \cdot n_m}{d\mu_H} = -0.00341$$

$$a = 3.80 \cdot 10^{-7} \text{ cm.}$$

These values of  $a$  thus appear as a result of acid binding experiments. They agree as well as could possibly be expected with that found on the basis of osmotic measurements, viz.

It should not, indeed, be quite exact, as we have disregarded  $1/q$ , which may assume a considerable value relative to  $w$ . Taking this term into consideration, then we obtain:

$$\frac{1}{2(w + 1/q) \cdot 0.4343} \cdot c_k = -\frac{dc_k \cdot n_m}{d\mu_H}.$$

Inserting in this equation  $c_k = 2.802 \cdot 10^{-5} \cdot o$ , calculated from the molecular weight, we obtain, for the case a)  $o = 31.7$

$$w + \frac{1}{q} = 0.1013.$$

Inserting now the value  $2.21 \cdot 10^{-7}$  from the osmotic measurements in the expression for  $w$ , this quantity is calculated to 0.0505, and  $q$  will be expressed by:

$$q = \frac{1}{0.1013 - 0.0505} = \text{ca } 20.$$

This figure for the number of acid and base groups sounds plausible enough in itself, but it must be admitted that it is in reality hardly more than a curiosity.

The whole of this calculation is open to an objection which it is necessary to refer to here. It might perhaps be imagined that the straight lines in Fig. 3 were only a fortunate sample section from a simple dissociation curve; that we had happened to hit on a rectilinear part of such a curve; the dissociation curve for sodium acetate — acetic acid is practically rectilinear at the point where the mean valency is about  $-1/2$ . This, however, is not the case for egg albumin, as will be seen from Table 4.

Table 4.

раН	o	c <sub>k</sub>	c <sub>k</sub> · n <sub>m</sub>	n <sub>m</sub>
4.043	31.7	$8.88 \cdot 10^{-4}$	0.00824	+ 9.28
4.109	—	—	0.00742	+ 8.36
4.199	—	—	0.00653	+ 7.35
4.290	—	—	0.00555	+ 6.25
4.396	—	—	0.00441	+ 4.97
4.552	—	—	0.00295	+ 3.32
4.731	—	—	0.00111	+ 1.25
4.867	—	—	0	0

It will here be seen that the mean valency within our limits varies from 0 to 9, so that a simple dissociation, at any rate in the sense here meant, is out of the question.

*Carlsberg Laboratory, June 1924.*

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# STUDIES ON CASEIN.

BY

KINSUKE KONDO.

(KIOTO, JAPAN).

The following investigations into the qualities of Casein were carried out during a period of study at the Carlsberg Laboratory from the summer of 1922 to the winter of 1923. The work was planned on a somewhat broad foundation, inter alia to give the writer an opportunity of becoming acquainted with the methods of physical chemistry, and could not be finally completed at all points during the relatively short time allotted. The writer therefore most cordially thanks Professor S. P. L. Sørensen, head of the Chemical Department at the Laboratory, for going through the present manuscript and arranging for its publication, in a revised and somewhat abbreviated form, in the papers issued by the Carlsberg Laboratory.

The writer also wishes to thank Prof. Sørensen heartily for kind assistance and guidance in the course of the work itself, as well as Fru Prof. Sørensen, Hr. cand. polyt. Jessen-Hansen and Hr. cand. polyt. K. Linderstrøm-Lang for advice, instruction and kindly criticism as colleagues.

## 1. The Casein employed.

The casein I used was prepared by Kahlbaum according to the Hammarsten method, and all investigations were carried out with the same preparation. The water-content, determined partly by drying in vacuo for 24 hours at 80°, partly by standing for three months in a sulphuric acid exsiccator, was 9.43 %. The ash content and nitrogen content of the dried casein were 0.15 % and 15.46 % respectively. (For the method of nitrogen determination, see S. P. L. Sørensen, Studies on Proteins<sup>1</sup>). The factor by which the quantity of nitrogen in the casein must be

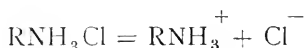
<sup>1</sup> Comptes-rendus du Lab. Carlsberg 12. (1917).

multiplied to give the corresponding weight of dry casein is thus  $100 \cdot 15.46 = 6.47$ .

## 2. Casein Chloride.

### A. General Survey.

When a protein at the isoelectric point is dissolved in a dilute acid, e. g. HCl, the concentration of the hydrogen ions in the acid decreases. As originally presumed by S. Bugarszky and L. Liebermann<sup>1)</sup> in their investigations with albumin and hydrochloric acid, this is due to the fact that part of the acid combines chemically with the added protein. The compound thus effected should be analogous with that of ammonia and hydrochloric acid, the protein being presumed to contain the amino-groups  $-\text{NH}_2$  which with HCl form  $-\text{NH}_3\text{Cl}$ . That portion of the protein  $\text{RNH}_2$  which has united with HCl should then have the formula  $\text{RNH}_3$ , in a water solution dissociated according to the formula:



On measuring the concentration of both hydrogen ions and chlorine ions in the protein solution, with varying content of protein and acid, it should therefore be possible to determine both the quantity of acid thus bound and the degree of dissociation which has taken place.

From this point of view, J. Loeb<sup>2)</sup> for instance, has investigated the concentration of hydrogen ions and of chlorine ions in hydrochloric acid solutions, with and without gelatine, and found that the concentration of chlorine ions in the hydrochloric acid solution was not affected by the addition of gelatine, which suggests that the gelatine chloride was completely dissociated.

As regards the value of such a chemical view in contrast to the multifarious adsorption theories which have been formulated from time to time, reference may be made to S. P. L. Sørensen, *Studies on Proteins* (I. c.) and K. Linderstrøm-Lang: *On the Ionisation of Proteins*<sup>3)</sup> where various features are noted for the elucidation of the problem. It will here suffice briefly to mention some works specially dealing with the ionisation of casein.

<sup>1)</sup> Arch. ges. Physiol. **72**, 51. (1898).

<sup>2)</sup> »Proteins and the theory of colloidal behavior«, New York and London. (1922).

<sup>3)</sup> Compt. rendus du Lab. Carlsberg **15**, Nr. 7. (1924).

Among earlier works may be noted those of L. L. van Slyke and E. B. Harts<sup>1)</sup> and L. L. van Slyke and D. D. van Slykes<sup>2)</sup><sup>3)</sup> where the writers, in researches with casein and inorganic acids, chiefly by measurements of conductivity, came to the conclusion that the binding of casein to acid was an adsorption phenomenon. This result, in the opinion of the writer, simply means that the binding of casein to acid is of so complex a character that it cannot be dealt with theoretically on the basis of simple premises. T. B. Robertson<sup>4)</sup> has, by detailed experimental work, arrived at the conclusion that casein and acid really combine chemically, but that the dissociation of the compound in water does not proceed in a simple manner, complex ions being formed by complicated dissociations of the protein molecule. It is perhaps somewhat doubtful whether such an assumption can be taken as generally valid, but it must be admitted as possibly serviceable in the explanation of many phenomena. But Pauli<sup>5)</sup> for instance, does not share Robertson's view, and from works by his collaborators K. Manabe and J. Matula<sup>6)</sup> it appears that salts of proteins with acids are dissociated in the same way as ammonium chloride.

Among other more recent works may be noted that of B. Bleyer and Seydel<sup>7)</sup>, and an investigation by D. J. Hitchcock<sup>8)</sup>, based on simultaneous measurement of the concentration of hydrogen ions and of chlorine ions in a protein chloride solution in the same way as employed by Loeb.

### *B. Solubility of Casein in dilute Hydrochloric Acid.*

There are a few experimental works<sup>4)</sup> on this subject, especially based on highly diluted acids, but we cannot go further into these here. I will merely mention that J. Loeb<sup>9)</sup> has found that 1 g. of finely pulverised casein dissolves after 24 hours in 100 cc of water, containing 6—30 cc 0.1 n HCl; i. e. 1 g casein dissolves

<sup>1)</sup> Amer. Chem. Journ. **33**, 461. (1905).

<sup>2)</sup> Ibid. **38**, 383. (1907).

<sup>3)</sup> Journ. Biol. Chem. **4**, 259. (1908).

<sup>4)</sup> »The physical Chemistry of the Proteins«. Longmans, Green and Co. (1918).

<sup>5)</sup> »Kolloidchemie der Eiweisskörper« **1**, 45. (1920). Dresden and Leipzig.

<sup>6)</sup> Biochem. Zeitschr. **52**, 369. (1913).

<sup>7)</sup> Ibid. **128**, 48 (1922).

<sup>8)</sup> Journ. of Gen. Physiol. **5**, 3, 383 (1923).

<sup>9)</sup> Journ. of Amer. Chem. Soc. **44**, 1930. (1922).

in 100 cc hydrochloric acid with concentration from 0.006 to 0.03 normal. At 0.005, a small precipitate remains, and at concentrations below this, or above 0.03, only a part of the casein dissolves. D. J. Hitchcock came to the same result.

Having previously ascertained, by formoltitration of casein solutions with varying quantities of hydrochloric acid, that the casein was not decomposed in the hydrochloric acid, I proceeded to make a series of experiments for investigating the solubility of casein in hydrochloric acid on the following lines:

1 or 1.5 g not dried casein was placed in a 100 cc measuring flask together with 30—40 cc distilled water free from carbon dioxide. After standing for 2 hours, with frequent shaking, the desired amount of hydrochloric acid was introduced, as highly diluted as possible, and water added to make 100 cc. The flask was then placed in a thermostat at 18° and left to stand for 24 hours, being shaken as frequently as possible to prevent the precipitate from settling. After this, the precipitate was centrifuged out from the solution, which was also filtered, and the amount of nitrogen in the solution determined. The results are shown in Table 1 and graphically in Fig. 1, where the abscissa is the no. of cc 0.1 n HCl added to 100 cc, and the ordinate the quantity of casein expressed in

Table 1.

HCl cc 0.1 n i 100 cc Solution	Total Casein (not dried) (Prec. + Solution) g pr. 100 cc Solution	Casein-nitrogen diss. in g pr. 100 cc Solution
4	1	0.0295
5	—	0.0500
6	—	0.0690
7	—	0.0950
8	—	0.1282
8.5	—	0.1378
9	—	0.1405
9.5	—	0.1405
10	—	0.1405
8	1.5	0.1005
9	—	0.1242
10	—	0.1523
11	—	0.1808
12	—	0.1972
13	—	0.2039
14	—	0.2078
14.5	—	0.2099
15	—	0.2104

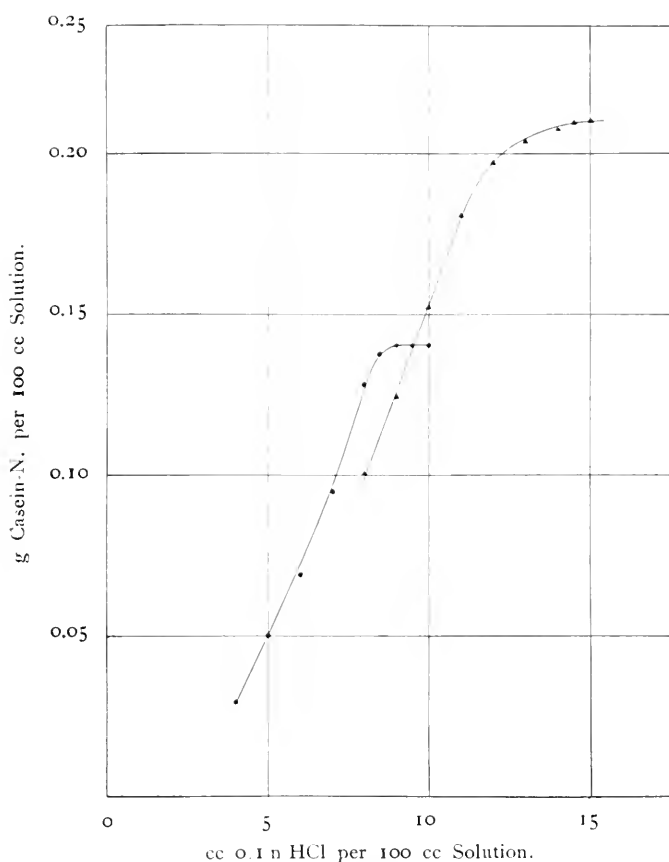
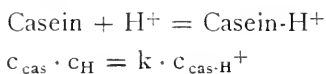


Fig 1.

g casein-nitrogen in 100 cc solution. From this we can obtain the weight of dry casein dissolved by multiplying by the factor 6.47 (see p. 2).

On studying Table I and Fig. 1 we notice, in the first place, that the solubility of the casein increases with the quantity of acid added, until all the precipitate is dissolved and the curve runs parallel with the abscissa. That this rise in the solubility must be due to an increasing ionisation of the casein is beyond all doubt, and since the curve is very nearly a straight line, it was natural to endeavour to explain the results by using the following ionisation formula



from which  $s$ , the solubility of the casein, is obtained:

$$s = c_{\text{cas}} + c_{\text{cas} \cdot \text{H}^+} = c_{\text{cas}} (1 + c_{\text{H}}/k). \quad (1)$$

Then, calling the concentration of acid added  $c'$ , we must have:

$$c_{\text{H}} = c' - c_{\text{cas} \cdot \text{H}^+} = c' - c_{\text{cas}} \cdot c_{\text{H}}/k$$

$$c_{\text{H}} = \frac{c'}{1 + c_{\text{cas}}/k} = K \cdot c' \quad (2)$$

where  $c_{\text{cas}}$  can be regarded as constant. Inserting this value for  $c_{\text{H}}$  in (1) we obtain:

$$s = k' + k'' \cdot c' \quad (3)$$

$s$  thus becomes a linear function of the concentration of hydrochloric acid.

There is, perhaps, the more reason to make such a calculation, since Edwin J. Cohn and L. Hendry<sup>1)</sup> in determinations of the solubility of casein in weak sodium hydroxide found a similar proportionality, and used it for a similar calculation. The actual position however, is doubtless more complicated than this. As will be seen from the figure, the curve for 1 g casein and that for 1.5 g casein (precipitate + solution) do not coincide, as they naturally should if the precipitate, the undissolved casein, remained unchanged throughout all the concentrations of hydrochloric acid here employed. The solubility evidently decreases — at the same concentration of hydrochloric acid — when the quantity of precipitate increases, and it is natural to suppose that the casein might contain some impurity capable of binding the acid and thus diminishing the solubility, the more precipitate, i. e. impurity, there was present.

In order further to investigate this point, the casein was therefore subjected to the following purifying process:

To 50 g. casein was added 1000 cc water, and then, slowly and with energetic stirring, 1500 cc 0.02 n NaOH, a quantity of base sufficient to dissolve all the casein without bringing the hydrogen ion concentration down below  $p_{\text{H}} = 8$ . The casein solution thus obtained was filtered, and the calculated quantity of 0.02 n HCl added, thus precipitating the casein again. With the filtrate from the precipitated casein (Filtrate I) analyses were made giving total nitrogen, ammonia nitrogen and formol-titratable nitrogen, and the concentration of hydrogen ions was determined. To the precipitated and carefully washed casein

<sup>1)</sup> Journ. of Gen. Physiol. 5. 521 (1923).

were then added water and 0.02 n NaOH, the whole being filtered and precipitated again with hydrochloric acid. The filtrate from this casein (Filtrate II) was then treated as before. Table 2 shows the results of the analyses.

Table 2.

	Filtrate I.	Filtrate II.
Volume	3420 cc	2700 cc
Total-N (mg)	146.0	13.2
Ammonia-N —	a trice	0
Formoltr.-N	—	0
pH	5.01	6.05

The casein thus twice re-precipitated was then used for determination of solubility, the quantity of precipitate being varied, and pH determined at the same time. The results are shown in Table 3, which also gives, for purposes of comparison, the results of similar experiments with non-purified casein.

Table 3.

cc 0.1 n HCl in 100 cc Solution	g Casein per 100 cc Solution (Prec + Sol.)	Solubility g Casein-N per 100 cc Solution	pH
Non-purified Casein			
6	1.0	0.0712	3.08
-	1.5	0.0628	3.30
-	3.0	0.0470	3.66
-	4.0	0.0410	4.00
Purified Casein			
6	1.0	0.0997	3.08
-	1.5	0.0886	3.08
-	3.0	0.0492	3.22

This table reveals again the peculiar phenomenon, for purified and non-purified casein alike, that the solubility of the casein increases the greater the quantity of precipitate in contact with the solution. Moreover, the purified casein has apparently a higher degree of solubility than the ordinary, in complete contradiction, as it would seem, of the fact that Filtrate II contains far less casein nitrogen per unit of volume than Filtrate I.

It is not easy to elucidate this feature completely on the basis of the present material. In the first place, it is possible

that any impurity present in the casein might not be eliminated by the process described; and further, that the hydrochloric acid might in some way or other be bound by the precipitate, thus effecting a distribution of the acid between the precipitate and the solution. The former possibility is rather slight. As regards the second, other features, e. g. the power of casein to bind not only hydrogen ions (acid binding) but also chlorine ions, in an aqueous solution (see p. 17) and especially the investigations by L. L. van Slyke, D. D. van Slyke and E. B. Harts, quoted above (pag. 3) that the precipitation of a sparingly soluble chloride, or in other words, an adsorption of HCl to the precipitate, may provide the explanation of the peculiarity in question. As the surface condition of a freshly precipitated preparation and of a dried preparation must necessarily be greatly dissimilar, the difference in solubility of the purified and non-purified casein on the basis of this view seems less incomprehensible. But if it be only a difference of this character which lies at the root of the divergent qualities in the two kinds of casein, we may with certainty presume that, as the analyses also show, the trade casein used really was pure but that extensive experiment is needed to elucidate the solubility of casein in hydrochloric acid. Hr. cand. polyt. K. Linderström-Lang, of the Carlsberg Laboratory, is engaged on a continuation of these investigations.

### *C. Dissociation of Casein Chloride.*

As mentioned in the introduction, simultaneous measurement of the concentration of hydrogen ions and concentration of chlorine ions in casein solutions affords a possibility for determining the ionisation of casein in hydrochloric acid solutions. As hydrochloric acid is a strong electrolyte, and as we shall, in what follows, have occasion to consider the influence of strong electrolytes, and also of salts, on the behaviour of casein, it may not be out of place here briefly to set forth the theoretical foundation on which recent views concerning the character of strong electrolytes are based, and the results arrived at, mainly through the work of G. N. Lewis, N. Bjerrum and J. N. Brønsted<sup>1</sup>).

<sup>1</sup> As to Literature see S. P. L. Sørensen and K. Linderström-Lang: Compt. rend. Lab. Carlsberg **15**, Nr. 6 (1924).



As we know, van't Hoff's application of the gas laws to solutions leads to the simple expression:

$$P = RTc, \quad (4)$$

where  $P$  is the osmotic pressure and  $c$  the concentration of the substance dissolved.

This law has been found to hold good with a fairly high degree of accuracy for dilute solutions of non-electrolytes, whereas in the case of electrolytes, characteristic divergencies appear, which are, in general, comprised in the empiric quantity  $i$ , dependent on  $c$ , giving (4) the form:

$$P = iRTc. \quad (5)$$

According to the classical dissociation theory, these divergencies were explained, as we know, by the suggestion that a fraction,  $\alpha$ , of the electrolyte was dissociated into ions, thus rendering the concentration higher than the stoichiometrical concentration. Assuming, for the sake of convenience, that it is a monovalent, binary electrolyte we have to deal with, then  $i$  will be determined by

$$i = 1 + \alpha.$$

Since  $\alpha$  is given by the mass action equation

$$\frac{c^2 \alpha^2}{c(1-\alpha)} = k_c \quad (6)$$

where  $k_c$  is the dissociation constant, and therefore approaches 1 when  $c$ , the stoichiometrical concentration, approaches 0, the connection between  $i$  and  $c$  is thus given,  $i$  being always less than 2, and approaching this value with increasing dilution.

Equation (6), and therewith the whole of this interpretation of  $i$ 's variation with  $c$  has been successfully applied to the weak, i. e. slightly dissociated electrolytes; this, however is not the case with the strong electrolytes. With these, it has been found necessary to proceed in quite a different way to arrive at results agreeing with those of experiment.

The classical dissociation theory, as we have noted, regards the decrease of  $i$  with the concentration ( $i$  at infinite dilution is 2, the electrolyte completely dissociated) as a result of increasing association of the positive and negative ions to neutral molecules. In contrast to this, Bjerrum now supposes that the strong electrolytes are completely dissociated, not only at infinite dilution, but also at finite concentrations, (e. g. 0.01—0.1 n). As then the number of particles on dilution of a given quantity of electrolyte does

not alter, there must be other causes to account for the alteration of the ratio  $P/c = iRT$  with  $c$ , and the Milner-Bjerrum theory seeks the main cause of this alteration in the electric forces whereby the electrically charged ions act on one another. Since these forces are, on the average, of an attractive character, it will be understood that as the ions, with increasing concentration, approach one another more and more nearly, the forces in question will more and more prevent them from acting as free particles, which is a condition for the validity of the gas laws as applied to the solution (i. e.  $P/c$  constant). And we understand also, that  $P/c$  must decrease with increasing concentration. More precisely expressed, the suppositions are as follows:

1) At infinite dilution  $c_0$ , the ions of a strong electrolyte (monovalent, binary) are so far removed one from another that the electrostatic forces between the ions are infinitesimal. These forces are given in Coulomb's law:

$$K = \frac{e_1 \cdot e_2}{r^2}$$

where  $r$  is the distance between the charges  $e_1$  and  $e_2$ , and it is at once seen that at an infinite distance, the force will be 0. As, moreover, no association takes place at infinite dilution, when the electrolyte is completely dissociated, the gas laws hold good without reserve, and  $P$  is determined by

$$P = RT \cdot 2 c_0, \quad (7)$$

the concentration of the ions being twice as great as the stoichiometrical concentration.

2) At finite concentrations, it is no longer possible to disregard the electric forces. There is, however, still no association to neutral molecules, but the ions can no longer be regarded as free particles, and the osmotic pressure cannot therefore be determined by the gas laws. According to Bjerrum, we set

$$P = RT \cdot 2 c f_0, \quad (8)$$

where  $f_0$ , the osmotic coefficient, is an expression of the reduction in osmotic pressure occasioned by the inter-ionic forces.  $f_0$ , which from the foregoing is equal to  $i/2$ , is therefore, in diluted solutions, always less than 1, and approaches this value with increasing dilution, (7) being valid for infinite dilution.

Quite empirically it has been found that the following expression used by Bjerrum:

$$1 - f_0 = k \cdot \sqrt[3]{c} \quad (9)$$

fairly adequately shows the variation of  $f_0$  with the concentration, and the most recent theoretical works (Debye-Hückel)<sup>1)</sup> support the views of Bjerrum, so that there can now hardly be any doubt as to their correctness, though an expression such as (9), which Ghosh, in a manner not altogether unexceptionable, has endeavoured to deduce theoretically, on the basis of Debye and Hückel's more exact calculations, for highly diluted solutions must be replaced by another:

$$1 - f_0 = k \cdot \sqrt{c} \quad (10)$$

I will not enter into these theoretical considerations here, but in the simple calculations which occur in the following, will take (9) as the basis, this equation being valid for a fairly extensive range of concentrations (0.005 n to 0.1 n).

Like the osmotic pressure and thus also the energy obtainable by reversibly, e. g. using a semi-permeable membrane, diluting the electrolyte, so also the free energy of the ions is diminished owing to the inter-ionic forces. For infinitely diluted electrolyte solutions we can write

$$F = RT \ln c_0 + j, \quad (11)$$

where  $j$  is a constant independent of the concentration, but this is not permissible with finite concentrations. Here we can, with Lewis, write:

$$F = RT \ln a + j \quad (11a)$$

$a$  is here called the activity of the ions, and is, owing to electric forces, less than  $c$ , the concentration of the ions. The quantity  $f = a/c$  is called the activity coefficient, and is an entirely similar quantity to  $f_0$ . It is 1 when the dilution is infinite, and less than 1 at finite concentrations. By the aid of thermodynamic methods we can find a connection between  $f$  and  $f_0$ :

$$1 + c \frac{d \ln f}{dc} = f_0 + c \frac{df_0}{dc}, \quad (12)$$

<sup>1)</sup> Physik. Zeitschrift, **24**, 185 (1923).

an expression first formulated by Bjerrum. On comparing (9) with this equation, we obtain:

$$\begin{aligned} \ln f &= -4k \cdot \sqrt[3]{c} \text{ or} \\ \log f &= -k' \cdot \sqrt[3]{c}. \end{aligned} \quad (13)$$

As already mentioned, the assumptions of the classical dissociation theory hold good approximately for the weak electrolytes, and it is also beyond doubt that the ions of these are to a high degree associated to neutral molecules; acetic acid, for instance, is very little dissociated into ions. For the equilibrium between hydrogen ions, acetate ions and neutral acetic acid molecules, we must therefore, when the concentration of ions in the acetic acid solution is extremely low, have the following:

$$c_H \cdot c_{Ac-} = k \cdot c_{Ac}. \quad (14)$$

If, on the other hand, the concentration of ions is finite, e. g. in a strong acetic acid solution, or in one to which has been added some salt, e. g. KCl, having no ion in common with the acetic acid, then, owing to the thermodynamic foundation of the law of mass action, and the part played by  $F$  in its derivation,  $a$ , the activity, must take the place of  $c$  in (14):

$$a_H \cdot a_{Ac-} = k \cdot a_{Ac}. \quad (15)$$

(15) now applies, of course, with thermodynamic exactitude. Inserting then  $f_H = a_H/c_H$  etc., we obtain:

$$\frac{c_H \cdot c_{Ac-}}{c_{Ac}} = k \cdot \frac{1}{f_H \cdot f_{Ac-}}, \quad f_{Ac} \text{ being, owing to the uncharged character}$$

of the acetic acid molecule, 1 in diluted ion solutions, and  $f_H$  and  $f_{Ac-}$ , the activity coefficients for the single ions, in the same way as the activity coefficient for the electrolyte, being determined by (13)

$$\begin{aligned} -\log f_H &= k_H \cdot \sqrt[3]{c_t} \\ -\log f_{Ac-} &= k_{Ac-} \cdot \sqrt[3]{c_t} \end{aligned}$$

where  $c_t$  is the total concentration of ions in the solution.

The writer does not suggest that these indications afford any profound insight into this activity theory. The field involved is far too extensive for this. Out of regard for what follows however, it will be useful to have laid down the main lines beforehand.

## a) Determination of chlorine ion activity.

For the determination of the activity of the chlorine ion I made use of the following element:



The electric potential of such an element must, according to (IIa), be determined by:

$$E = \frac{RT}{F} \ln \frac{a_x}{a_{0.1}} = 0.0577 \log \frac{a_x}{a_{0.1}} = 0.0577 (\log a_x - \log a_{0.1}) \text{ at } 18^\circ,$$

where E is the potential of the element,  $a_x$  the unknown chlorine ion activity in the experimental liquid,  $a_{0.1}$  the activity of the chlorine ions in the 0.1 n potassium chloride solution. Since the concentration of chlorine ions in the 0.1 n potassium chloride solution is 0.1, the potassium chloride being completely dissociated, it is possible to determine  $\text{pa}_{\text{Cl}} = -\log a_x$  in the experimental liquid when  $f_{0.1} = a_{0.1}/0.1$  is known, seeing that in such case

$$\text{pa}_{\text{Cl}} = \frac{-0.0577 (\log 0.1 + \log f_{0.1}) - E}{0.0577} = \frac{E_0 - E}{0.0577}, \quad (16)$$

$E_0$  being determined by

$$E_0 = -0.0577 (\log 0.1 + \log f_{0.1}) = 0.0577 (1 - \log f_{0.1}).$$

In order to determine  $f_{0.1}$ , I proceeded as follow. For the experimental liquids in Element (I), I used potassium chloride solutions of varying concentrations from 0.01 n to 0.1 n, E being, as before, determined by

$$E = 0.0577 \log \frac{a_x}{a_{0.1}} = 0.0577 \log \frac{c_x \cdot f_x}{0.1 \cdot f_{0.1}} = 0.0577 \left( \log \frac{c_x}{0.1} + \log \frac{f_x}{f_{0.1}} \right).$$

Inserting here  $-\log f_x = k \cdot \sqrt[3]{c_x}$ , it is evidently possible to determine k, since

$E = 0.0577 (\log \frac{c_x}{0.1} + k (\sqrt[3]{\frac{0.1}{0.1}} - \sqrt[3]{c_x}))$ , the only unknown quantity in which is k.

$$k = \frac{1}{\sqrt[3]{\frac{0.1}{0.1}} - \sqrt[3]{c_x}} \left( \frac{E}{0.0577} - \log \frac{c_x}{0.1} \right) \quad (17)$$

Table 4 shows k calculated according to (17),  $\sqrt[3]{\frac{0.1}{0.1}} - \sqrt[3]{c_x}$  being called d.

Table 4.

$c_x$	$E$	$E/0.0577$	$\log \frac{c_x}{0.1}$	$d \cdot k$	$d$	$k$
0.01	-0.0522	-0.9047	-1.0000	+0.0953	0.2486	0.383
0.01	-0.0532	-0.9219	-1.0000	0.0781	0.2486	0.314
0.02	-0.0362	-0.6274	-0.6990	0.0716	0.1927	0.372
0.02	-0.0368	-0.6377	-0.6990	0.0613	0.1927	0.318
0.03	-0.0270	-0.4679	-0.5229	0.0350	0.1533	0.360
0.04	-0.0205	-0.3553	-0.3979	0.0426	0.1221	0.349
0.05	-0.0151	-0.2617	-0.3010	0.0393	0.0957	0.422
0.06	-0.0112	-0.1941	-0.2218	0.0277	0.0727	0.381
0.07	-0.0077	-0.1335	-0.1549	0.0214	0.0520	(0.412)
0.08	-0.0048	-0.0832	-0.0969	0.0137	0.0365	(0.375)
0.10	-0.0002	—	—	—	—	—
0.10	+0.0001	—	—	—	—	—

From this table we can calculate the mean value  $k = 0.362$  disregarding the two last experiments, with 0.07 and 0.08 n KCl, where  $E$  is rather uncertain, and we can therefore find  $f_{0.1}$ , as

$$\log f_{0.1} = -0.362 \cdot \sqrt[3]{0.1} = -0.1680,$$

and determine  $E$ , since

$$E_0 = 0.0577 (1 - \log f_{0.1}) = 0.0577 \cdot 1.1680 = 0.0674.$$

The measurements were carried out in a thermostat at  $18^\circ$ . The electrode vessel (of the usual type) was thoroughly washed and dried, and in it was placed, first a little pure mercury (twice redistilled) and thereafter a calomel paste prepared from calomel electrolytically precipitated according to the Lipscomb Huletts method<sup>1)</sup> by repeated washing with the experimental liquid. Finally, the experimental liquid was poured in. The electrodes were measured after standing for one day.

By means of this determination of  $E_0$  we can now, measuring the potential of the element (I), determine the chlorine ion activity of an arbitrary experimental liquid, e. g. a casein solution. Calling the potential  $E_{Cl}$  we obtain, according to (16)

$$p_{Cl} = \frac{0.0674 - E_{Cl}}{0.0577} \quad (18)$$

at  $18^\circ$ .

<sup>1)</sup> Journ. Amer. Chem. Soc. **38**, 22 (1916).

## b) Determination of hydrogen ion activity.

Theoretical considerations and experimental results lead in precisely the same way as with determination of chlorine ion activity to the expression:

$$p_{aH} = \frac{E_H - 0.3348}{0.0577 + 0.0002(t - 18.0)^1} \quad (19)$$

where  $p_{aH} = -\log a_H$ ,  $a_H$  is the hydrogen ion activity in the solution and  $E_H$  the potential of the following element:

Hg, HgCl, 0.1 n KCl/3.5 n KCl/Exp. liquid,  $H_2$ , Pt.

The measurements were made with the Hasselbalck shaking electrode in an air thermostat, introducing the correction term for temperature in (19).

## c) Casein solutions.

For determination of the ionisation of the casein in hydrochloric acid, solutions were prepared with 1 g. casein and 1.5 g. casein per 100 cc solution, and different quantities of hydrochloric acid. Here, however, to avoid possible decomposition of the casein, care was taken not to add too great a surplus of HCl beyond the quantity required to dissolve the quantities of casein employed (see Table 1). The hydrochloric acid interval we have to reckon with is thus very narrow, but on the other hand, it is possible that the accuracy of the measurements is thereby increased. The activity of hydrogen ions and that of chlorine ions were measured, i. e.  $p_H$  and  $p_{aCl}$ , and from these two quantities, we can calculate both the hydrogen ion and the chlorine ion concentrations, as

$$p_{aCl} = -\log a_{Cl} = -\log c_{Cl} - \log f_{Cl} = -\log c_{Cl} + 0.362 \sqrt[3]{c_t} \\ -\log c_{Cl} = p_{aCl} - 0.362 \sqrt[3]{c_t}$$

and

$$p_{aH} = -\log a_H = -\log c_H - \log f_H = -\log c_H + 0.2 \sqrt[3]{c_t} \\ -\log c_H = p_{aH} - 0.2 \sqrt[3]{c_t}$$

where the equation  $\log f_H = -0.2 \cdot \sqrt[3]{c_t}$  is taken as the basis, this being the equation on which (19) is itself based (see Bjerrum &

<sup>1)</sup> See for instance Bjerrum and Gjaldbæk. D. kgl. Vet. og Landbohøjskoles Aarskrift 1919, pag. 73 and E. Schreiner, Zeitschr. f. an. u. allg. Chem. **115** (1921). In a paper recently published by S. P. L. Sørensen and K. Linderstrøm-Lang, Comptes-rendus du Lab. Carlsb. **15**, Nr. 6, (1924) the value 0.3357 calculated on the basis of an extended formula by Bjerrum is used instead of 0.3348 in (19).

Gjaldbæk l. c.) in the same way as (18) is formulated on the basis of  $\log f_{Cl} = -0.362 \cdot \sqrt[3]{c_{Cl}}$ .

Table 5a.

1 g not dried Casein in 100 cc solution.

Conc. of HCl				
$Q = c_{HCl} \cdot 10^3 \dots\dots$	9.0	9.5	10.0	10.5
Det. of $p_{aH}$ .				
Temperature ....	19.8	18.9	19.2	19.2
$E_H$ (Volts) .....	0.4990	0.4922	0.4874	0.4840
$p_{aH} \dots\dots\dots$	2.827	2.719	2.634	2.558
$0.2 \cdot \sqrt[3]{c_{HCl}} \dots\dots\dots$	0.042	0.042	0.043	0.044
$-\log c_{HCl} \dots\dots\dots$	2.785	2.677	2.591	2.514
$P = c_H \cdot 10^3 \dots\dots$	1.641	2.104	2.564	3.062
$Q = c_{HCl} \cdot 10^3 \dots$	9.0	9.5	10.0	10.5

Det. of  $p_{aCl}$ .

Temperature ....	18	18	18	18
$E_{Cl}$ (Volts) .....	-0.0516	-0.0599	-0.0587	-0.0573
$p_{aCl} \dots\dots\dots$	2.236	2.206	2.185	2.161
$0.362 \cdot \sqrt[3]{c_{HCl}} \dots\dots\dots$	0.075	0.077	0.078	0.079
$-\log c_{Cl} \dots\dots\dots$	2.161	2.129	2.107	2.082
$R = c_{Cl} \cdot 10^3 \dots\dots$	6.902	7.430	7.816	8.279
$Q - P \dots\dots\dots$	7.359	7.396	7.436	7.438
$R - P \dots\dots\dots$	5.261	5.326	5.252	5.217
$x = R - P/Q - P \dots$	0.715	0.720	0.706	0.701
$q \cdot 10^4 \dots\dots\dots$	2.10	2.07	2.18	2.22

Table 5b.

1.5 g Casein in 100 cc solution.

$Q = c_{HCl} \cdot 10^3 \dots\dots$	14.5	15.0	15.5	16.0
Det. of $p_{aH}$ .				
Temperature ....	17.6	17.6	17.5	18.3
$E_H$ (Volts) .....	0.4847	0.4808	0.4773	0.4745
$p_{aH} \dots\dots\dots$	2.602	2.533	2.473	2.419
$0.2 \cdot \sqrt[3]{c_{HCl}} \dots\dots\dots$	0.049	0.049	0.050	0.050
$-\log c_H \dots\dots\dots$	2.553	2.484	2.423	2.369
$P = c_H \cdot 10^3 \dots\dots$	2.799	3.281	3.776	4.276



Table 5b (continued).

Det. of  $\text{pa}_{\text{Cl}}$ .

Temperature . . . .	18	18	18	18
$E_{\text{Cl}}$ (Volts) . . . . .	— 0.0500	— 0.0490	— 0.0481	— 0.0471
$\text{pa}_{\text{Cl}}$ . . . . .	2.033	2.017	2.002	1.984
$0.362 \cdot \sqrt[3]{C_{\text{HCl}}}$ . . . . .	0.088	0.089	0.090	0.091
$-\log c_{\text{Cl}}$ . . . . .	1.947	1.928	1.912	1.893
$R = c_{\text{Cl}} \cdot 10^3$ . . . .	11.30	11.80	12.25	12.79
$Q - P$ . . . . .	11.70	11.72	11.72	11.72
$R - P$ . . . . .	8.501	8.520	8.474	8.514
$x = R - P/Q - P$ . .	0.727	0.727	0.723	0.726
$q \cdot 10^4$ . . . . .	2.13	2.13	2.17	2.14

In Tables 5a and 5b, the measurements with the casein solutions are noted. The hydrochloric acid concentration is inserted for  $c_{\text{Cl}}$ . This is perhaps not correct, as some of the chlorine is bound to the protein and the majority of the hydrogen. As we have no means of determining the valency of the protein ions, it seems to me hardly worth while to introduce any correction for the quantity of acid bound in any complex manner, especially as the correction in any case would be very small. Similarly also, no correction has been introduced for the volume of the disperse phase, as this lies well within the limit of experimental error. The values noted for  $\text{pa}_{\text{H}}$ ,  $E_{\text{H}}$ ,  $\text{pa}_{\text{Cl}}$ ,  $E_{\text{Cl}}$  are the mean of three parallel measurements. With the terms introduced in the tables ( $Q = C_{\text{HCl}} \cdot 10^3$ ,  $P = C_{\text{H}} \cdot 10^3$  og  $R = C_{\text{Cl}} \cdot 10^3$ )  $R - P/Q - P = x$  evidently means the degree of dissociation of the casein chloride, expressed in the usual way.  $q$  is the number of complex-bound gram-equivalents of chlorine ions per g. casein (not dried). It will be noted that both  $x$  and  $q$  appear to be independent of the concentration of hydrogen ions.

The power of casein to bind chlorine ions together with hydrogen ions, i. e.  $\text{HCl}$ , which we in the solubility determinations have regarded as the cause of the peculiar conditions here occurring, is thus also apparent in these acid-binding experiments. With regard to the manner in which this binding takes place, however, it is difficult to say anything in the present state of our knowledge of proteins generally.

*D. Osmotic Pressure and Membrane Potential.*

There is an overwhelming abundance of literature concerning the osmotic pressure of protein solutions, and since Donnan put forward his theory as to the influence exerted by a non-diffusible ion on the distribution of diffusible ions on both sides of a semi-permeable membrane, numerous investigators, among them especially J. Loeb<sup>1)</sup> have gone deeply into the questions of osmotic pressure and membrane potential, as also that of the connection between these and electrolyte concentration or concentration of hydrogen ions.

The Donnan theory has been of great importance in the development of the theoretical chemistry of proteins. Viewed in the light of the activity theory, the facts are in reality very little altered. The electric potential difference  $E_M$  is merely determined here by:

$$E_M = \frac{RT}{F} \ln \frac{a'}{a''} \quad (20)$$

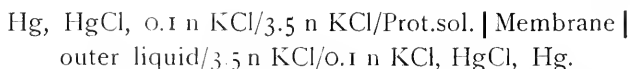
where  $a'$  and  $a''$  represent the activity in a state of equilibrium of one of the monovalent diffusible ions on the one side and on the other side of the membrane. And if we take sodium chloride as the only diffusible electrolyte, Donnan's simple equation

$$c'_{Na} \cdot c'_{Cl} = c''_{Na} \cdot c''_{Cl}$$

is altered accordingly to

$$a'_{Na} \cdot a'_{Cl} = a''_{Na} \cdot a''_{Cl} \quad (21)$$

The measurement of the membrane potential is effected by means of the following element:



where the protein solution, in this case the casein solution, contains, in addition to the protein, also the diffusible ions with activities associated with the corresponding activities in the outer liquor by means of equations such as (20) and (21).

We obtain, of course, with thermodynamic certainty, the same  $E_M$  by electrometric measurement of the activity of one of the diffusible ions, for instance, the hydrogen ion, in both protein solution and outer liquor, and insertion of the activities thus ob-

<sup>1)</sup> Journ. of Amer. Ch. Soc. **44**, 1929 (1922). Journ. of Gen. Physiol. **5**, 665, 693 (1923). Proteins and the theory of colloidal behavior (loc. cit.).

tained in (20). But the direct measurement of the membrane potential presents so great advantages in practical respects, that it is to be preferred where possible.

The apparatus I employed for measurement of  $E_M$  is shown in Fig. 2.

The collodium cap A, which served as the semipermeable

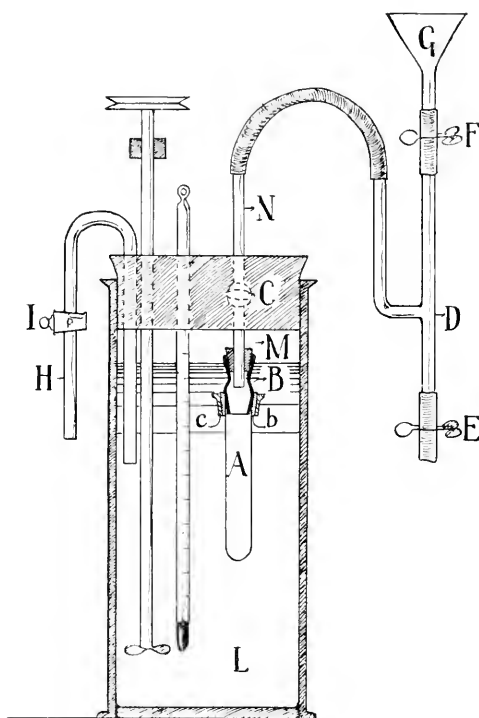


Fig. 2.

membrane, is placed on a short, slightly conical glass tube B, and made to fit tightly by means of rubber tubing and a glass ring c (cf. S. P. L. Sørensen: Studies on Proteins I. c. pag. 296). B is provided at the top with a rubber stopper, M, through which is passed a glass tube N with a cock C, whereby the space below can be shut off. This glass tube is connected by a rubber tubing with the T-tube D from the top of which again a rubber tubing with a clip F is carried to a funnel G, the lower end of the T-tube terminating in a rubber tubing with clip E. The collodium cap is placed, together with a stirrer, a thermometer, and the glass U tube H with tap I, in the cylinder glass L containing the

outer liquor to be used. The collodium cap, and the system of tubes CDEFG are filled with the casein solution in the following manner: First of all, the collodium cap and tube B are filled completely by means of a pipette. Then some of the casein solution is poured into the funnel G, and F and C opened, while E is kept closed. This fills the entire system to the lower end of N. C is now closed, and the rubber stopper M placed carefully into the aperture of B and pressed home; C is again opened for a moment, to remove the surplus pressure from A, and then closed for the remainder of the experiment. M is paraffined, to prevent any electric conduction through the stopper. Finally, the collodium cap is rinsed on the outside with the outer liquor and placed in L in the outer liquor there, the tube H being also filled with outer liquor.

When the membrane potential is to be measured, the rubber tube with clip E is removed, and on opening F, D is filled completely to its lower end, and a couple of drops of casein solution run off; similarly, by opening I, a little of the outer liquor is drawn off through the lower aperture of H, and the two tubes, H and D are put down into two vessels containing 3.5 n KCl. This brings them into connection with two reciprocally measured 0.1 n calomel electrodes, which provide the two poles of the element<sup>1)</sup>.

By means of this apparatus, I measured the membrane potential for casein solutions composed precisely as mentioned in Section C, and with outer liquors only containing hydrochloric acid at a suitable initial concentration. The potential was determined several times a day, and for several days in succession, until equilibrium was established, and the potential attained a constant value, which as a rule took place 36–48 hours after the commencement of the experiment. Finally, the activity of hydrogen ions, both in the inner and the outer liquor, was measured.

Further, the osmotic pressure of the same casein solutions was measured by means of the method worked out chiefly by J. A. Christiansen (see *Studies on Proteins*, I. c). Here also the activity of hydrogen ions was measured in the inner and outer liquor after the experiment.

The results are shown in Tables 6a, 6b, 7a, 7b and Figures 3 and 4.

<sup>1)</sup> Of course, neither C nor I should be greased with vaseline.

Table 6a.

1 g Casein in 100 cc solution.

At the beginning.

$c_{\text{HCl}} \cdot 10^3$ inside.....	9.0	9.5	10.0	10.5
$c_{\text{HCl}} \cdot 10^3$ outside .....	7.5	8.0	8.5	9.0

At equilibrium.

$p_{\text{aH}}$ inside .....	2.423	2.369	2.366	2.313
$p_{\text{aH}}$ outside .....	2.204	2.178	2.133	2.118
$E_{\text{M}}$ (Volts) measured.....	0.0127	0.0101	0.0099	0.0096
$E_{\text{M}}$ calculated from $p_{\text{aH}}$ ..	0.0126	0.0110	0.0134	0.0113

Table 6b.

1.5 g Casein in 100 cc solution.

At the beginning.

$c_{\text{HCl}} \cdot 10^3$ inside.....	14.5	15.0	15.5	16.0
$c_{\text{HCl}} \cdot 10^3$ outside .....	12.0	12.5	13.0	13.5

At equilibrium.

$p_{\text{aH}}$ inside .....	2.261	2.200	2.160	2.132
$p_{\text{aH}}$ outside .....	2.013	1.979	1.979	1.946
$E_{\text{M}}$ (Volts) measured.....	0.0150	0.0120	0.0109	0.0109
$E_{\text{M}}$ calculated from $p_{\text{aH}}$ ..	0.0147	0.0131	0.0107	0.0111

In Fig. 3, the directly measured  $E_{\text{M}}$  in millivolts is set off as ordinate, and the  $p_{\text{aH}}$  of the inner liquid as abscissa.

Table 7a.

1 g Casein in 100 cc solution.

At the beginning.

$c_{\text{HCl}} \cdot 10^3$ inside .....	9.0	9.5	10.0	10.5
$c_{\text{HCl}} \cdot 10^3$ outside .....	7.5	8.0	8.5	9.0

At equilibrium.

$p_{\text{aH}}$ inside .....	2.456	2.334	2.320	2.267
$p_{\text{aH}}$ outside .....	2.203	2.178	2.145	2.118
$E_{\text{M}}$ calculated from $p_{\text{aH}}$ ..	0.0148	0.0092	0.0103	0.0088
Osmot. press. (cm water) ..	14.46	13.59	13.03	12.23

Table 7b.

1.5 g Casein pr. 100 cc solution.

At the beginning.				
$c_{\text{HCl}} \cdot 10^3$ inside.....	14.5	15.0	15.5	16.0
$c_{\text{HCl}} \cdot 10^3$ outside .....	12.0	12.5	13.0	13.5
At equilibrium.				
$p_{\text{aH}}$ inside .....	2.261	2.199	2.141	2.119
$p_{\text{aH}}$ outside .....	2.013	1.979	1.972	1.949
$E_{\text{M}}$ calculated from $p_{\text{aH}}$ ..	0.0146	0.0130	0.0100	0.0101
Osmot. press. (cm water) ..	18.71	18.10	16.70	15.71

In Fig. 4, the  $p_{\text{aH}}$  of the inner liquor is taken as abscissa and the osmotic pressure as ordinate.

It will be seen from these tables and curves that both osmotic pressure and membrane potential decrease with increasing activity of hydrogen ions. This could perhaps seem to be in opposition to what one might expect, as  $E_{\text{M}}$  should rise higher the more there is formed of the non-diffusible casein ion, the farther we move from isoelectric reaction in the acid direction. It must be borne in mind, however, that other factors must have to be reckoned with. For instance, it is not only the concentration of hydrogen ions, but also that of the chlorine ions which is altered by varying the quantity of hydrochloric acid; we alter, indeed, the whole ion concentration of the casein solution. Furthermore it has been shown by the investigations of J. Loeb (se p. 3) that a surplus of hydrochloric acid precipitates the casein anew after it has been completely dissolved at lower concentrations of hydrochloric acid, which seems to suggest a twofold action of the hydrochloric acid, an ionising effect proceeding from the hydrogen ion, and a salting-out effect proceeding from the chlorine ion, which has altogether a specific effect on casein. The correctness of this assumption is confirmed by some purely qualitative experiments which I have made as to the effect of NaCl on dissolved casein chlorine. A perfectly clear solution of casein in hydrochloric acid turned quite cloudy on adding NaCl to the concentration 0.01 N, and at 0.1 N, large quantities of casein were precipitated.

But apart from such suppositions, we are again unaware as to the activity effect of the large complex casein ions on formation of the membrane potential, and what "osmotic coefficients"

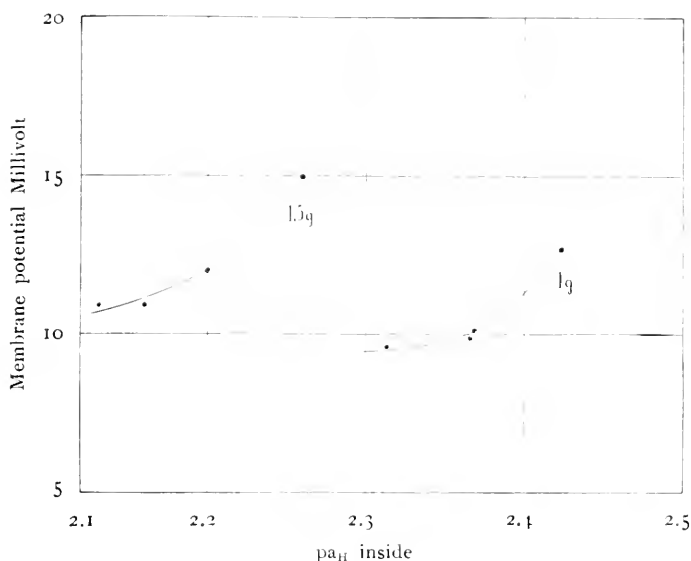


Fig. 3.

we may have to reckon with in dealing with the osmotic pressure. In Section C we have reckoned that the ions contributed

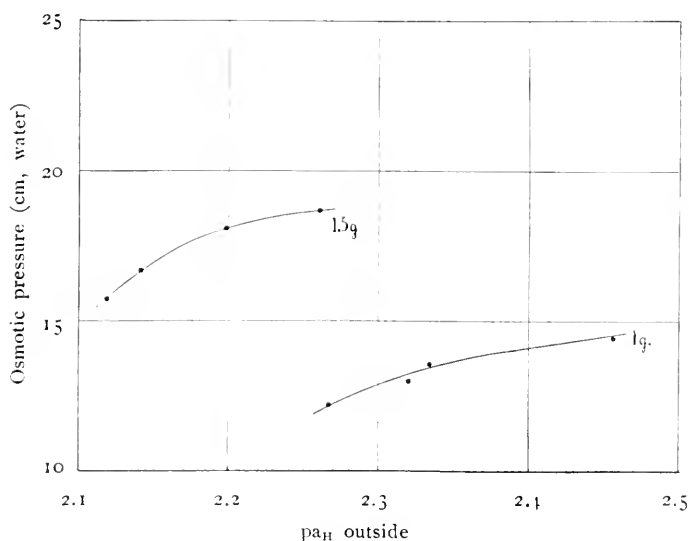


Fig. 4.

by the hydrochloric acid, with their electric charges, acted as if the hydrochloric acid was free and the hydrogen ions not bound

to the casein to polyvalent ions of complex composition. This is not correct, but we cannot reckon otherwise at present, nor can we say anything definite as to the conditions which determine the alteration of  $E_M$  and the osmotic pressure with the activity of the hydrogen ions.

### 3. Alkali Caseinate.

#### *A. General Observations.*

In chapter 2, I described some investigations with casein chloride. In the present chapter, more or less corresponding measurements of ionisation, osmotic pressure and membrane potential of sodium caseinate (and potassium caseinate) will be given. The writer will not here present any detailed survey of extant literature, but it may be of interest, in regard to what follows, to mention here one or two of the most important researches in this field.

E. Laqueur and O. Sackur were the first to investigate, in a well known work<sup>1)</sup> the conductivity of caseinates. The solution they employed was neutral as regards phenolphthalein, so that 1 g casein would equivalent to 0,881 millimol NaOH. If we here assume that the casein is present as a monovalent ion, its molecular weight will be 1135, but it must be noted that this supposition is almost certainly incorrect, and the writers in question, on grounds into which we need not enter here, came to the conclusion that the casein must be at least tetravalent and possibly 5—6-valent.

T. B. Robertson<sup>2)</sup> has also investigated this question. Starting with certain assumptions which have already been referred to here (p. 3) he arrives at the conclusion that alkali caseinate does not, on dissociation, give off alkali ions, but is dissociated into complex polyvalent ions containing alkali in a non-dissociable form.

W. Pauli and his collaborators<sup>3)</sup> made measurements of conductivity and likewise interpreted them in a simple manner. According to these investigations, the casein should, at neutral reaction, be tribasic, and the valency increase with decreasing

<sup>1)</sup> Beitr. Chem. Physiol. Pathol. **3**, 193 (1903).

<sup>2)</sup> Journ. of phys. Chem. **11**, 437 og 522 (1907) — **12**, 473 (1908) — **14**, 377, 601 (1910).

<sup>3)</sup> Biochem. Zeitschr. **70**, 489 (1915), *ibid.* **99**, 219 (1919).



concentration of hydrogen ions, whereas an increase of the hydrogen ion concentration causes the formation of a complex salt,  $\text{Na}_3\text{-Casein}$  [Casein]. For the rest, as regards, the ionised condition of proteins, I would refer to the work by K. Linderstrom-Lang quoted on p. 2, where the question as to alteration in the valency of the protein with the  $\text{pH}$  is dealt with in association with both adsorption theory and law of mass action. Such simple views of the constitution of protein solutions as those based on the Ostwald law of dilution, for instance, can hardly survive the test of experiment; we may, indeed, assert that measurements of conductivity, in the present state of our knowledge as to theory of solutions, are the most difficult of all physico-chemical measurements to deal with, with the sole exception of viscosity measurements.

Nevertheless, it is possible that the few measurements I have made, partly of viscosity and partly of conductivity in casein solutions, may be of some interest.

#### *B. Viscosity of Solutions of Casein in NaOH and KOH.*

Many proteins are decomposed in alkaline solution. Pauli maintains that proteins, on the addition of even small amounts of base, undergo a change in course of time, which in his opinion cannot be ascribed to decomposition of the proteins; this however, is hardly certain.

The experiments I have made here distinctly suggest that the casein solutions in alkali are constant — at any rate within certain limits of time — and do not alter, for instance, either their viscosity or their conductivity in course of time, as long as the quantities of base added are small.

I prepared the solutions in the following manner (see also p. 4) 25 g of casein was placed with a small amount of pure conductivity water in a 500 cc measuring flask. After shaking for 2—3 hours, 50 cc 0.2 N NaOH, diluted as far as possible, was dropped in very slowly, with lively shaking. Water was then added up to the mark. After standing for 24 hours, the solution was centrifuged, analysed for casein, placed in an ice chest, and served thereafter as stock solution for the experiments, the liquids used for these being prepared from the stock solution by adding water and such other materials as required. The constant and

reproducible results I have obtained with a stock solution of this sort, despite the fact that experiments extended over a period of weeks, are a guarantee that there is really no alteration taking place at low temperature. As we shall now see, the same is shown by viscosity measurements at 18° with experimental liquids prepared from the same.

The viscosity measurements were carried out in the usual manner with an Ostwald viscosimeter in a thermostat at  $18^{\circ} \pm 0.05$ . Some parallel determinations of the specific gravity of the casein solutions were made with a Sprengel pyknometer, with correction for the buoyancy of the air.

Table 8.

Conc. of KOH	0.005	0.006	0.007	0.008	0.009	0.010	0.011
Nr. Viscosim. .	III	II	III	II	III	II	III
Time from the beginning of the experiment	Time for outflow, sec.						
20 Min.	68.8	62.3	70.7	65.4	74.6	68.6	78.2
60 —	68.8	62.3	70.7	65.2	74.6	68.3	77.5
100 —	do.	do.	do.	65.4	74.6	68.3	77.0
150 —	do.	do.	do.	do.	74.2	67.8	77.0
200 —	do.	do.	do.	do.	74.0	67.7	76.5
20 Hours	do.	do.	do.	64.4	73.2	—	—
24 —	—	—	—	do.	do.	66.9*	75.2*
48 —	—	—	—	do.	do.	do.	do.

1.1 g Casein in 100 cc solution.

Table 8 shows the constancy of the viscosity with time. The solutions were prepared by diluting the stock solution with water and varying quantities of 0.02 n KOH solutions, and it is clearly evident that the viscosity does not alter when the base added does not exceed, 0.007 n. With higher concentrations of the base, however, a slight fall in the viscosity is apparent, though it seems, after the lapse of some time, to attain a constant value. In experiments with larger quantities of base I have used this constant value, generally after 48 hours. The results are shown in Table 9.

\* 30 hours.

Table 9.

g Casein pr. 100 cc	D specific gravity 18°/4°	Time for outflow sec.	No. Viscmet.	$\eta$	$pa_H$
Sodium caseinate.					
4.388	1.01226	197.0	II	3.705	6.364
2.194	1.00510	110.4	III	1.840	6.476
1.097	1.00201	71.9	II	1.338	6.577
0.5485	1.00057	68.5	III	1.136	6.706
0.2743	0.99958	57.7	II	1.071	6.831
0.1371	0.99937	62.8	III	1.040	6.979
0.0686	0.99904	55.4	II	1.025	—
0.0343	0.99894	61.4	III	1.017	—

## Potassium caseinate.

4.388	1.01262	198.8	II	3.740	6.371
2.194	1.00561	110.7	III	1.846	6.473
1.097	1.00225	81.4	III	1.352	6.565
0.5485	1.00063	68.8	III	1.141	6.667
0.2743	0.99975	57.7	II	1.071	6.749
0.1371	0.99930	62.9	III	1.042	6.805
0.0686	0.99915	55.3	II	1.027	—
0.0343	0.99897	61.5	III	—	—

Table 9 gives measurements of specific gravity and viscosity of solutions with different concentration of casein, prepared by diluting the stock solution with water.  $\eta$  is the relative viscosity. Fig. 5 shows, graphically, the relation between casein concentration and viscosity. It will be seen from the tables how  $pa_H$  rises slowly with increasing dilution.

Table 10 and Fig. 6 show the relation between  $pa_H$  and viscosity at constant casein concentration. It will be noted — as Loeb has also shown — that the viscosity rises evenly with the  $pa_H$  up to 9, and then increases rapidly. This seems to suggest that the casein is decomposed here, which is confirmed by determinations of formol-titratable nitrogen.

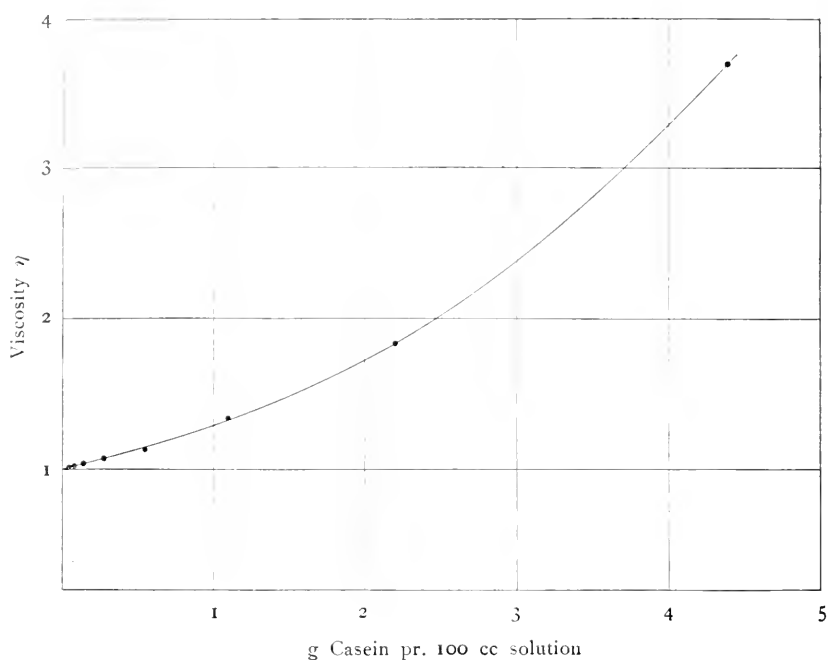


Fig. 5.

Table 10.

1.097 g Casein in 100 cc solution.

Conc. of KOH.	D	Time for outflow sec.	Nr. Viscimeter	$\eta$	$p_{aH}$
0.005	1.00063	68.8	III	1.141	6.667
0.006	1.00066	62.3	II	1.158	7.405
0.007	1.00069	70.7	III	1.173	8.466
0.008	1.00067	64.4	II	1.197	9.930
0.009	1.00071	73.2	III	1.214	10.473
0.010	1.00086	66.9	II	1.244	10.915
0.011	1.00083	75.2	III	1.248	10.956

It is very interesting to note, in this connection, that the alteration of  $p_{aH}$  with the addition of base decreases very markedly as soon as  $p_{aH}$  exceeds the value 10 (see curve II Fig. 6), which also might seem to suggest decomposition.

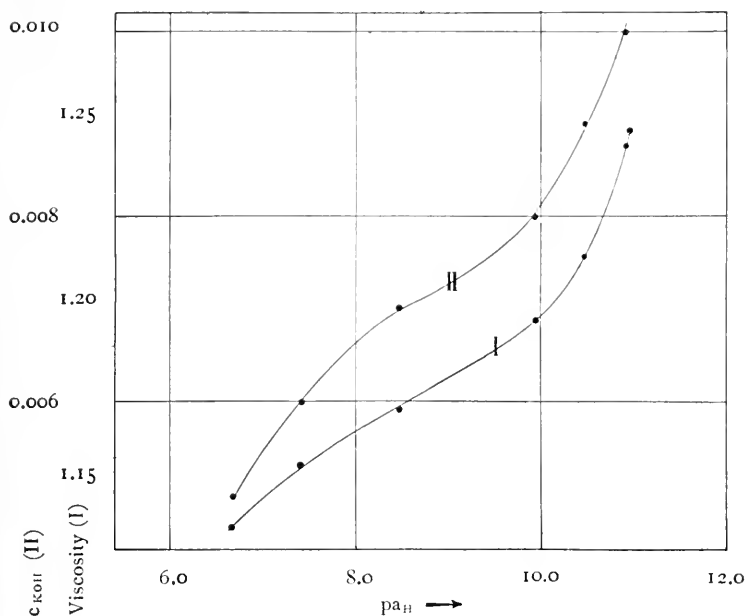


Fig. 6.

### C. Conductivity of Alkali Caseinate Solutions.

As described in Section B the  $pa_H$  in an alkali caseinate solution does not alter particularly on dilution, and we may therefore perhaps follow Pauli (l. c.) in comparing the alkali caseinate solution with a solution of a typical neutral salt. According to the activity theory, which we have briefly described in the foregoing, the inter-ionic forces greatly affect not only the osmotic pressure and the free energy of such salts in solution, but also

their conductivity. The quantity  $\alpha = \frac{\mu}{\mu_\infty}$  which, according to the classical dissociation theory, should indicate the degree of dissociation of the neutral salt becomes, presuming the electrolyte to be completely dissociated, merely an expression of the restriction imposed on the free mobility of the ions by the electric forces between them; a restriction which increases in proportion as the ions are nearer one another, i. e. the higher the concentration is. At infinite dilution, the ions are perfectly free, and  $\alpha$  is therefore 1; with increasing concentration  $\alpha$  decreases, and quite empirically, it has been found that an expression entirely analogous with (13) viz:

$$\log \alpha = -k |c|^{-1} \quad (22)$$

very well reproduces the results of the experiments.  $c$  is the concentration of the salt.

From a purely theoretical point of view, however, the matter is one of far greater difficulty than the corresponding calculation for  $f$  and  $f_0$ , where we are dealing with thermodynamic equilibrium and in a case such as the present, where further complications arise owing to the high viscosity of the casein solution even at low concentrations, the position is even more difficult to survey. A viscosity correction according to the following formula:

$$\alpha = \frac{\eta'}{\eta_\infty} \cdot \frac{\eta}{\eta_\infty}$$

or according to Washburn<sup>2</sup>):

$$\alpha = \frac{\eta'}{\eta_\infty} \cdot \left( \frac{\eta}{\eta_\infty} \right)^m \quad (m \text{ is about } 1)$$

cannot be applied in this case, because the variation of the viscosity is high and leads also to quite meaningless results. It is therefore not strange that an equation such as (22) should not agree with our results, though the high  $\alpha$  we find distinctly suggests that the alkali caseinate is a completely dissociated electrolyte.

Table II.  
Sodium caseinate.

g Casein in 100 cc sol.	$p_{H_2}$	$L \cdot 10^6$	V	$\eta'$	$\alpha$
4.388	6.364	86.61	50	43.30	(0.461
2.194	6.476	49.20	100	49.20	0.524
1.097	6.577	26.61	200	53.22	0.565
0.5485	6.706	14.65	400	58.60	0.623
0.2743	6.831	7.994	800	63.96	0.680
0.1371	6.979	4.184	1600	66.94	0.712
0.0686	—	2.267	3200	72.53	0.772
0.0343	—	1.229	6400	78.66	0.837)

<sup>1</sup> Ghosh: Journ. Chem. Soc. **113**, 449, 627, 707 (1918).

<sup>2</sup> Journ. Amer. Chem. Soc. **33**, 1461 (1911).

Table 12.

## Potassium caseinate.

g Casein in 100 cc sol.	$p_{\text{H}}$	$L \cdot 10^5$	V	$\mu$	$\alpha$
4.388	6.371	119.30	50	59.65	(0.519
2.194	6.473	65.67	100	65.67	0.571
1.097	6.565	35.44	200	70.87	0.616
0.5485	6.667	18.84	400	75.35	0.655
0.2743	6.749	10.11	800	80.91	0.704
0.1371	6.805	5.300	1600	84.81	0.737
0.0686	—	2.816	3200	90.11	0.784
0.0343	—	1.580	6400	101.11	0.879)

The results of the experiments are given in Tables 11 and 12, and Fig. 7. The solutions were prepared, as before, by dilution of the stock solution; the conductivity was measured by means of an apparatus from Hartmann & Braun, of Frankfurt a/M. The cell constant was 7.769 mho and the conductivity of the pure water used  $9.35 \cdot 10^{-7}$  mho. In the tables, L denotes the specific conductivity, V the dilution in litres per gram equivalent alkali, and  $\mu$  is the equivalent conductivity calculated after  $\mu = L \cdot 1000 \cdot V$ . The values for  $\alpha$  are calculated from directly extrapolated values of the conductivity at infinite dilution  $\mu_{\infty}$ :

For sodium caseinate . . . . . = 94

For potassium caseinate . . . = 115

and are consequently highly uncertain. They are therefore placed in parentheses, as they merely denote the order of magnitude of  $\alpha$ . On the other hand, the difference between the conductivity of potassium and sodium caseinate at the same dilution is of course liable only to experimental errors, and serves to show with certainty that the alkali caseinate is really dissociated into alkali ions and casein ions of some sort and another, as pointed out by Pauli. Thus the difference 22.5 between  $\mu_{\text{Pot-Cas}}$  and  $\mu_{\text{Sod-Cas}}$  at  $V = 6400$  is in good agreement with the value found by Kohlrausch for the difference between mobility of the potassium ion and that of the sodium ion.

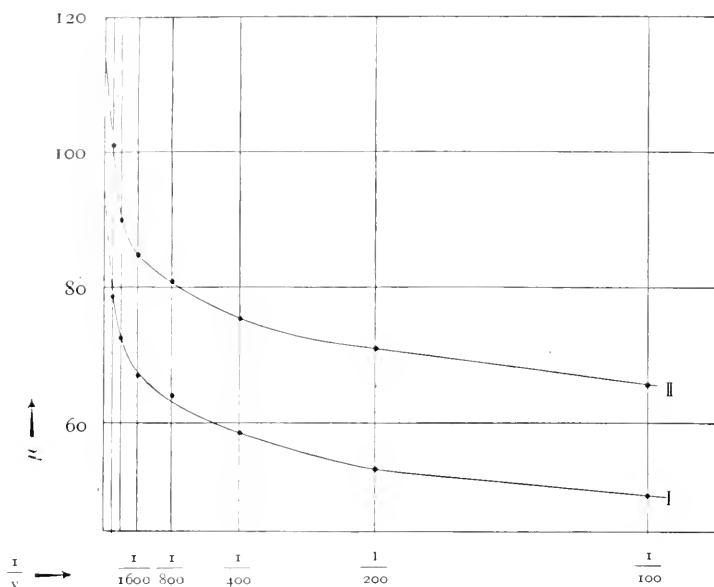


Fig. 7

*D. Influence of Sodium Chloride on the Properties of Solutions of Casein in Sodium Hydroxide.*

a. Activity of the hydrogen ion and its relation to base concentration and sodium chloride concentration.

For these investigations, a stock solution was prepared as follows: 16 g casein was placed with a little water in a 500 cc measuring flask, and there dissolved in diluted sodium hydroxide, answering to 40 cc 0.2 n NaOH, after which the flask was filled up to the mark. After standing for 24 hours at indoor temperature, the solution was centrifuged and analysed for casein.

For the experiments, I took 25 cc of this solution and diluted with water containing varying quantities of NaCl and NaOH to 50 cc, after which the concentration of hydrogen ions was determined by means of the Hasselbalck electrode. The results are set out in Table 13 and graphically in Fig. 8, where the abscissa is the measured  $p_{a_H}$ , and the ordinate the concentration of NaCl.



Table 13.  
0.6635 g casein in 50 cc solution.

$c_{\text{NaOH}}$	$c_{\text{NaCl}}$	$p_{\text{H}}$	Numbers on Fig. 8
$8 \cdot 10^{-3}$	0.0	7.192	I
—	0.1	6.725	
—	0.5	6.440	
—	1.0	6.343	
$8.5 \cdot 10^{-3}$	0.0	7.343	II
—	0.1	6.892	
—	0.5	6.613	
—	1.0	6.503	
$9.0 \cdot 10^{-3}$	0.0	7.487	III
—	0.1	7.065	
—	0.5	6.777	
—	1.0	6.688	
$9.5 \cdot 10^{-3}$	0.0	7.639	IV
—	0.1	7.310	
—	0.5	6.944	
—	1.0	6.879	

This series of experiments has some features of interest, the casein here for instance, behaving in the same way as acetic acid on the addition of salt, a point which may be explained by the activity theory. If we write the dissociation equation of acetic acid after (15) (p. 12) as

$$a_{\text{H}} \cdot a_{\text{Ac}^-} = k_{\text{E}} \cdot a_{\text{Ac}}$$

and remember that  $f_{\text{Ac}}$  can be taken as equal to 1, we obtain:

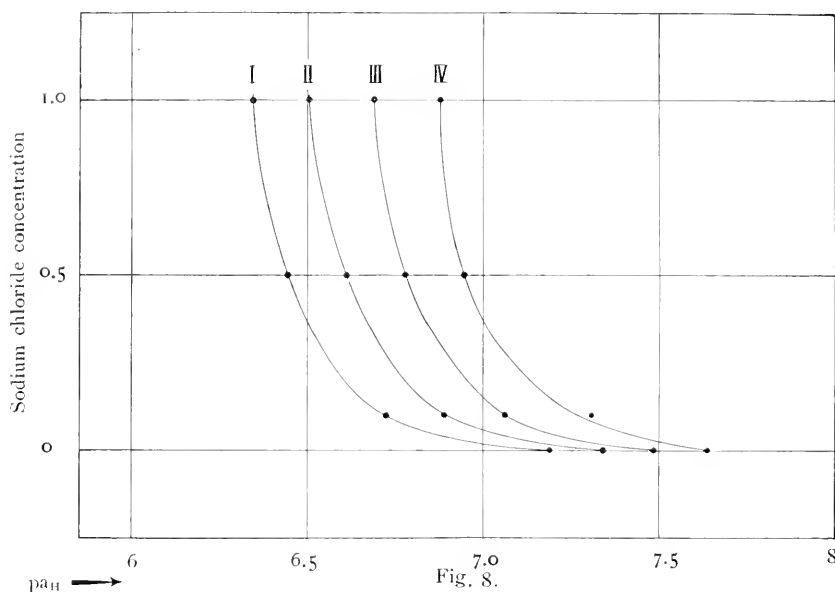
$$a_{\text{H}} = k_{\text{E}} \cdot \frac{c_{\text{Ac}^-}}{c_{\text{Ac}}} \cdot \frac{1}{f_{\text{Ac}^-}}. \quad (23)$$

If we are now at a concentration of hydrogen ions at which both  $c_{\text{Ac}}$  and  $c_{\text{Ac}^-}$  are high, as for instance  $a_{\text{H}} = k_{\text{E}}$  in a not too highly diluted (0.1 n) solution of acetic acid and sodium hydroxide, we can approximately, altogether qualitatively, reckon  $c_{\text{Ac}}$  and  $c_{\text{Ac}^-}$  as constant, when we add an electrolyte, as for instance NaCl. We can therefore write (23) as

$$a_{\text{H}} = \frac{b}{f_{\text{Ac}^-}},$$

where  $b$  is a constant. As  $f_{Ac-}$  after the formula  $\log f_{Ac-} = -k_{Ac-} \sqrt[3]{c}$  decreases with increasing concentration of salt,  $a_H$  must increase, as the experiments also show.

Quite qualitatively, this view agrees with our measurements of casein, since  $pa_H$  decreases, and  $a_H$  increases with increasing salt concentration. And we cannot, indeed, expect more. If



the casein is polyvalent — as it doubtless is — then (23) must be altered according to the valency we attribute to the casein, and the position is further complicated by the fact that  $f_{Cas-}$ , which must take the place of  $f_{Ac-}$ , will also have to include the valency,  $f$  being an expression of the forces between the electric charges. We have for the sake of simplicity up to now only considered monovalent electrolytes, and I will not here go into the calculations which might possibly be made with our problem in this respect, but merely observe that it does not seem as if the alteration in  $pa_H$  can be explained solely by electric effect on the casein ions, at any rate in the sense in which such effect is regarded in the activity theory.

#### b. Osmotic pressure and membrane potential.

In the following will be found some experimental results of measurements of osmotic pressure and membrane potential in sodium caseinate solutions, prepared in a similar manner to those

described in Section a., but with different concentrations of sodium chloride (see Table 14).

Table 14.

Numbers of experiments g casein in 100cc sol.....	1	2	3	4	5
	2.194	2.194	2.194	2.194	2.194
At the beginning.					
$c_{\text{NaOH}}$ inside....	0.02	0.02	0.02	0.02	0.02
$c_{\text{NaCl}}$ inside....	0.001	0.002	0.01	0.02	0.10
Viscosity inside.	1.937	1.878	1.690	—	1.495
$c_{\text{NaCl}}$ outside....	0.001	0.002	0.01	0.02	0.10
At equilibrium.					
Osm. press. (cm water) .....	84.86	52.46	19.65	—	6.16
$p_{\text{H}}$ mean .....	6.530	6.486	6.293	6.236	5.930
$E_{\text{M}}$ (Millivolts) ..	-44.88	-27.11	-12.05	-6.80	-1.59

Table 14 and Figs. 9 and 10, where  $E_{\text{M}}$  is the membrane potential and the values given for  $p_{\text{H}}$  are mean values from those found by determination of osmotic pressure and membrane potential measurements, show the marked influence exerted by concentration of sodium chloride on both osmotic pressure, membrane potential and viscosity of the sodium caseinate solution, an influence which can certainly not be attributed solely to the parallel alteration in the  $p_{\text{H}}$ . This can be seen, for instance, by comparing the alteration in viscosity with  $p_{\text{H}}$  in Table 14 with the corresponding alteration in Table 10 where the ion concentration has not been altered as in these experiments. If we wish to form an idea as to what takes place, we can proceed in the following manner, as indicated by Linderstrøm-Lang<sup>1)</sup>.

We imagine a system as that given below:

Inner liquid	Outer liquid
Protein with mean valency $n$ (negative) <sup>2)</sup> and concentration $c_s$	
Corresponding sodium ion concentration $n \cdot c_s$	Sodium ion concentration $c_y$
Chlorine ion concentration $c_i$	Chlorine ion concentration $c_y$
Corresponding sodium ion concentration $c_i$	

Semipermeable membrane,

<sup>1)</sup> privately communicated.

<sup>2)</sup> cf. Linderstrøm-Lang l. c.

an arrangement answering to that originally given by Donnan. Assuming now that the concentration of hydrogen ions is slight in comparison with  $n \cdot c_s$  and  $c_i$ , and that we may, in the first approximation, set concentrations in place of activities in the

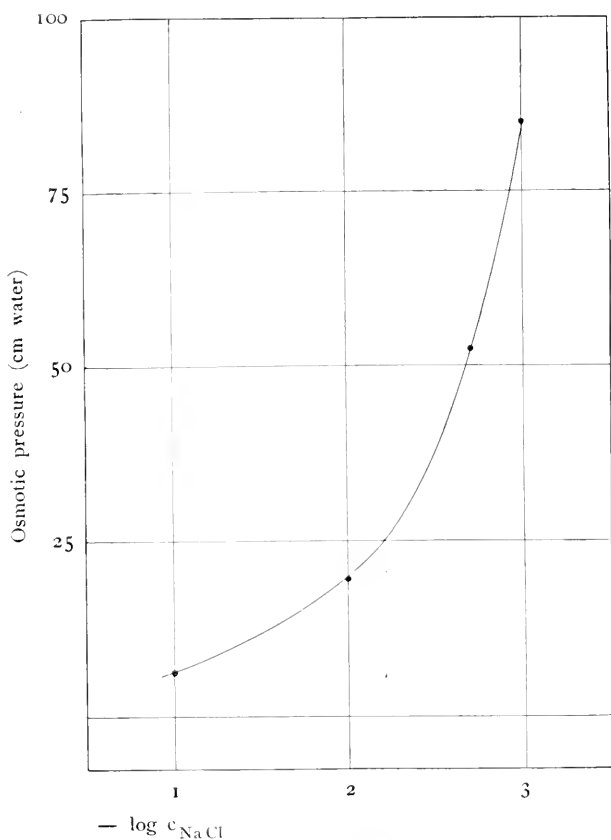


Fig. 9.

thermodynamic equations and also take all osmotic coefficients as = 1, we obtain, according to Donnan, the following 3 equations.

$$E_M = 0.0577 \cdot \log \frac{c_i}{c_y} \quad (\text{at } 18^\circ, E_M \text{ reckoned from left to right}) \quad (24)$$

$$\text{and} \quad E_M = 0.0577 \cdot \log \frac{c_y}{n \cdot c_s + c_i} \quad (25)$$

$$\text{and} \quad P = RT (c_s + n \cdot c_s + 2 c_i - 2 c_y), \quad (26)$$

$P$  being the osmotic pressure.

Combining (24) and (25) we obtain in a well-known manner

$$c_y^2 = c_i (n \cdot c_s + c_i) \text{ og } n \cdot c_s = \frac{c_y^2 - c_i^2}{c_i}$$

which, inserted in (26) gives

$$P = RT \left( c_s + \frac{(c_y - c_i)^2}{c_i} \right) \quad (27)$$

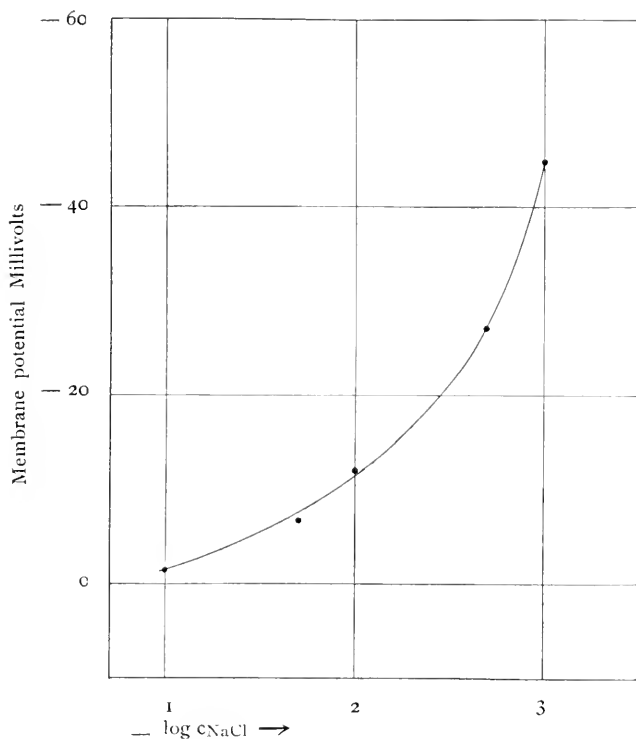


Fig. 10.

Combining this equation with (24) we obtain the following connection between  $P$ ,  $c_y$  and  $E_M$ :

$$P = RT (c_s + c_y \cdot 10^{E_M/0.0577} \cdot (10^{-E_M/0.0577} - 1)^2) \text{ ved } 18^\circ. \quad (28)$$

The results of a calculation with this formula of the values in Table 14 are shown in Table 15.

$c_y$  is determined by direct analysis in the outer liquid,  $c_s$  which we get by subtraction of the numbers in column 5 from the numbers in column 6 (Table 15) has thus values which are 0 or negative, which last must of course be due either to experimental error or to some error in the theory. But it must

Table 15.

RT =  $2.388 \cdot 10^4$  Liter · cm water pressure.

$c_y \cdot 10^3$	$\frac{E_M}{0.0577}$	$10^{E_M/0.0577}$	$(10^{E_M/0.0577} - 1)^2$	$\frac{P}{RT} - c_s$ (28)	$\frac{P}{RT}$ (meass.)
1.0	-0.778	0.167	25.0	$4.16 \cdot 10^{-3}$	$3.55 \cdot 10^{-3}$
1.91	-0.470	0.339	3.80	2.19 —	2.20 —
9.10	-0.209	0.618	0.301	2.14 —	0.823 —

be borne in mind that the potential and the osmotic pressure are not determined by the same experiment, and we must therefore reckon with a high experimental error, inter alia because the activity of hydrogen ions cannot be made exactly the same in two parallel series of experiments. The results are therefore not suited for determination of  $c_s$ , but they show, qualitatively, that the osmotic pressure which rises from the unequal distribution of the ions  $P - RTc_s$  (column 5) is of the same order of magnitude as  $P$  measured (column 6). From this point of view then, Table 15 seems to indicate a possibility of explaining the enormous alteration in osmotic pressure with salt concentration from such considerations, without necessarily having recourse to any assumptions as to the condensation of casein particles. It is very remarkable, though we cannot at present attach any special importance to the fact, that the two lowest concentrations of sodium chloride, where the calculation should probably be correct with a certain approximation, seem to show that the molecular weight of casein is very high. Before we can say anything certain, however, we must have a more accurate theory and more accurate experiments.

#### 4. Concluding Remarks.

The main results of the present work are as follows:

1°. Measurements of the solubility of casein in HCl revealed the peculiar fact that the solubility decreases — with the same concentration of HCl to begin with — as the quantity of precipitate in contact with the solution is increased. I have postponed the explanation of this feature until more comprehensive investigations have been made, but have just suggested that it should perhaps be sought in the capacity of casein to cause complex binding of chlorine ions both in the solution and together with

hydrogen ions in the precipitate, such a distribution of the HCl between precipitate and solution being well calculated to explain the experimental results.

2°. By measurement of hydrogen ion activity and chlorine ion activity in solutions of casein in hydrochloric acid, and reckoning back to concentrations after the activity theory, it was found that the casein in the solution binds per gr. abt.  $2 \cdot 10^{-4}$  gram equivalents of chlorine ions at the hydrogen ion activities used.

3°. Measurements of osmotic pressure and membrane potential in casein solutions containing varying quantities of hydrochloric acid serve mainly to confirm the investigations of J. Loeb, though the apparatus used may perhaps be useful as the basis for further development of the experiments in quantitative respects.

4°. The determination of viscosity and conductivity in alkali caseinate solutions confirms, on the one hand the investigations of J. Loeb and on the other those of W. Pauli. It was found however, in our experiments as contrasted with those of Pauli — that casein solutions do not alter their condition in course of time, as long as the quantity of base added is small.

5°. Determination of the base-binding power of casein on addition of NaCl and NaOH in varying quantities, revealed a rise in the activity of hydrogen ions with the concentration of sodium chloride with constant quantity of base. This is analogous for instance, to the behavior of acetic acid under similar conditions, and can at any rate in part, be explained by the activity theory.

6°. Measurements of osmotic pressure and membrane potential in sodium caseinate solutions reveal an extremely high degree of influence exerted by sodium chloride on both osmotic pressure and membrane potential. A brief reproduction is given of a theory for the relation between concentration of sodium chloride, osmotic pressure and membrane potential, a theory closely allied to that of Donnan, and with a very restricted sphere of validity, but nevertheless seeming to show that the molecular weight of casein is high.

*June 1924*

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# STUDIES ON PROTEINS

BY

S. P. L. SØRENSEN.

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## VII. ON THE COAGULATION OF PROTEINS BY HEATING<sup>1)</sup>.

BY

MARGRETHE AND S. P. L. SØRENSEN.

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The question as to coagulation of proteins by heating is of ancient date, and has been made the subject of numerous researches, and of speculation in like degree. Up to the present, no final and decisive answer to the question has been found, and I may say at once, I have none such to give you to-day. What I wish to put before you is merely the result of some experiments throwing light on a single phase of this question, which is one of great importance in the chemistry of proteins.

Coagulation consists of two distinct processes; a denaturation of the protein, and subsequent precipitation or flocculation of the denaturated protein. This has already been noted by W. E. Hardy<sup>2)</sup> and later by W. Pauli and H. Handovsky<sup>3)</sup> but this side of the question was first really made clear in the series of excellent works by Harriette Chick and C. J. Martin<sup>4)</sup>.

Chick and Martin have, as far as possible, investigated the two sections of the coagulation process separately, and have studied the importance of the salt concentration and the hydrogen ion concentration of the solution for both processes. I shall not here go into details concerning these investigations, or those of earlier writers on the subject. Two things, however, I wish to point out. Chick and Martin have shown that the denaturation,

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<sup>1)</sup> The present paper is mainly from the text of a lecture delivered before the New York section of the American Chemical Society (<sup>3</sup>/<sub>10</sub> 1924).

<sup>2)</sup> Journ. of Physiol. **24**, 182 (1899).

<sup>3)</sup> Beitr. zur Chem. Physiol. u. Pathol. **11**, 425 (1908).

<sup>4)</sup> Journ. of Physiol. **40**, 404 (1910), **43**, 1 (1911), **45**, 61 and 261 (1912);  
Resumé in Kolloidchemische Beihefte V, 49 (1913).

that is to say, the actual transformation of the protein, takes place more rapidly, and at a lower temperature, the higher the concentration of hydrogen ions — or, in the case of alkaline solutions, the concentration of hydroxyl ions is. They have also shown that the process of denaturation is a monomolecular process, as long as the concentration of hydrogen ions — or in alkaline solutions, of hydroxyl ions — is kept constant during denaturation. The precipitation of the denaturated protein, on the other hand, as has been shown by L. Michaelis and his collaborators, takes place more rapidly and more completely, the nearer the concentration of hydrogen ions approaches to the isoelectric reaction of the particular protein in question.

Experiments we have made at the Carlsberg Laboratory, independently of the work of Chick and Martin, and at about the same time, as well as quite recent researches by W.W. Lepeschkin<sup>1</sup>), confirm in all essentials Chick and Martin's results.

It will be natural, then to regard the latter part of the coagulation, — the precipitation, or flocculation, of the denaturated protein — as proceeding, at any rate in the main, from a de-charging of the electrically charged particles of denaturated protein, which, after denaturation, behaves like a typical suspensoid.

It is far more difficult to give an explanation, supported by experiments, of the first part of the coagulation process, the denaturation itself. It is at once obvious that we have here an alteration of the entire character of the protein. And it is highly probable that it is a question, not merely of an alteration in the degree of dispersity or similar physical qualities, but also of a true chemical change in the constitution or composition of the protein.

What then, can we imagine to be the nature of this process in the course of which the protein is denaturated?

Chick and Martin are of opinion that the denaturation is brought about by a reaction between the protein and water. They lay particular stress on the fact that dry, or nearly dry proteins can be heated to a degree far beyond the boiling point

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<sup>1</sup>) Kolloid-Zeitschrift **31**, 342 (1922), **32**, 42, 44 and 106 (1923); Biochem. Journ. **16**, 678 (1922).

of water without denaturation. But they do not venture to express any opinion as to whether, in denaturation, it is a question of hydration or dehydration; of a condensation or a decomposition of the protein molecule. They have endeavoured by means of formol-titrations to prove the existence of a hydrolysis, but without success. In these experiments, they used slightly alkaline solutions of crystallised egg albumin, or purified pseudoglobulin from horse serum. The results of these experiments are, as we shall see later on, not altogether in agreement with those of our own.

In the opinion of Lepeschkin also, everything seems to suggest that denaturation is an incipient, weak hydrolysis. He, however, makes no attempt to confirm this by experiment, but regards any demonstration of the hydrolysis by pure analysis as impossible, owing to the size of the protein molecules.

At the Carlsberg Laboratory, we have for many years had this problem under consideration. And in particular we have sought for some means of answering, with experimental proof, these two questions:

Firstly. Does the protein, on denaturation, give off any ammonia or other nitrogenous substances?

and Secondly. Does the protein give off or take up water during the process of denaturation?

I should like now to tell you something about the experiments we have made — for the most part with egg albumin — with a view to finding out an answer to these two questions, if possible.

### **A. Does the Protein, on Denaturation, give off any Ammonia or other nitrogenous substances?**

a) In a work published in 1910<sup>1)</sup> it was shown that an egg albumin solution, not subjected to any purification, but soured with hydrochloric acid to the optimal concentration of hydrogen ions for coagulation, required heating for a considerable time in a boiling water bath before the minimum value for nitrogen content of the filtrate was reached.

Table I shows the results of one series of our experiments.

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<sup>1)</sup> S. P. L. Sørensen and E. Jürgensen: Comptes-rendus du Lab. Carlsberg 10, 49 (1910).

TABLE 1.

Time of coagulation in hours	40 cc. solution					Concen- tration of hydrogen ions
	Consumption for neutral re- action with litmus paper a cc. of 0.2 n. sodium hydroxide solution	Consumption for faint red with phenolphthalein b cc. of 0.2 n. sodium hydroxide solution	Contained c mg. total nitrogen	Contained d mg. formol- titrable nitrogen	d % of c.	
	a	b	c	d		P <sub>H</sub>
Non-coagulated solution . . . .	1.86	2.08	181.7	8.34	4.59	4.70
1 hour . . . . .	0.13	0.15	11.83	0.48	4.06	5.41
3 — . . . . .	0.14	0.18	10.58	0.64	6.05	5.43
6 — . . . . .	0.15	0.20	10.49	0.98	9.34	5.53
12 — . . . . .	0.21	0.28	12.95	1.79	13.82	5.52
24 — . . . . .	0.22	0.40	17.18	2.94	17.11	5.60

It will be seen that the nitrogen content of the filtrate did not reach its minimum, and the coagulation thus its maximum, until after three or six hours' heating. But it will also be seen, that the filtrate's content of formol-titrable nitrogen — and more or less parallel with this, the consumption of sodium hydroxide for neutral reaction with litmus and phenolphthalein respectively — rise steadily with the period of heating, whether the coagulation be completed or not. The explanation must doubtless be, that we have here a dual process, partly a precipitation of coagulable proteins, and partly a hydrolysis, which results in a resolution of a part of the precipitated protein. It will further be seen, from the last vertical column but one in the table, that the quantity of formol-titrable nitrogen increases far more than the total nitrogen content of the filtrate. This is naturally explained by the fact that it is not only the precipitate, but in all probability, specially the substances present in the solution, which undergo further hydrolysis.

In this experiment, however, we had to deal with a mixture of the different proteins present in the white of hens' eggs, and

a clear answer to the question involved could hardly be expected. This would doubtless require investigations with solutions of pure proteins.

b) We have therefore resumed these studies, using for the protein solution a solution of egg albumin several times recrystallised by means of ammonium sulphate, and then completely freed from ammonia by washing the crystallised precipitate with a saturated solution of potassium and sodium sulphate, and then recrystallising again repeatedly, with the potassium-sodium sulphate solution as precipitant. Such a protein solution has the great advantage that the entire quantity of nitrogen present should be coagulable, so that determination of the ammonia-N and the total-N in the filtrates would give direct measurements for any decomposition taking place on coagulation.

In several of these experiments, we had to make a quantitative determination of very small amounts of nitrogen. The analytical methods used had therefore to be adapted to this purpose (ammonia determination after Nessler, and total nitrogen determinations after Kjeldahl-Nessler). Also, care had to be taken to use pure distilled water and reagents, particularly free from ammonia or as nearly so as possible. Any quantity of ammonia or other nitrogenous matter, however slight, in the chemicals used, had also to be determined by test experiments, and taken into account in the calculations. I will not go into details regarding the manner of these analyses or of the coagulation experiments. One point should be noted, however. The heating, that is to say, the denaturation itself, was carried out at various concentrations of hydrogen ions, but after cooling, the hydrogen ion concentration was brought to its optimum for flocculation,  $p_H = 4.7 - 4.8$ . This was done in the case of all the samples, by adding a suitable solution of acetic acid and sodium acetate. The heating was effected in conical flasks, covered with watch glasses and placed over, not down in, boiling water bath. The heating conditions were therefore not absolutely uniform for all the experiments, but nearly enough for the result to give a quantitative idea.

Table 2 shows the results of a series of experiments with different concentrations of hydrogen ions and different periods of heating. Most of the samples, which were only heated for one hour, were found not to have coagulated out completely. This

TABLE 2.

Each experiment contained 1.295 g. egg-albumin nitrogen.

Concentration of hydrogen ions in the solution after heating  pH	Time of heating							
	1 hour	3 hours	6 hours	12 hours	1 hour	3 hours	6 hours	12 hours
	Ammonia-N liberated in mg.				Total-N liberated in mg.			
abt. 3.8 . . . . .	0.18	0.52	0.67	1.12	0.19	0.73	3.26	6.30
abt. 4.4 . . . . .	— <sup>1)</sup>	0.44	0.58	0.98	— <sup>1)</sup>	0.58	1.17	3.89
abt. 4.65 . . . . .	— <sup>1)</sup>	0.54	0.60	0.98	— <sup>1)</sup>	0.63	1.39	2.98
abt. 4.9 . . . . .	— <sup>1)</sup>	0.50	0.65	0.99	— <sup>1)</sup>	0.61	1.60	2.47
abt. 6.1 . . . . .	— <sup>1)</sup>	0.83	0.94	1.46	— <sup>1)</sup>	1.45	2.45	3.28

was shown by the fact that the filtrates turned cloudy on adding strong sulphuric acid. The analysis results from these experiments, being too high, are not included in the table.

It will be clearly seen from the table that the decomposition was least in samples with hydrogen ion concentration approaching the isoelectric reaction of egg albumin ( $p_H = \text{abt. } 4.8$ ). Also, that the quantity of ammonia liberated was greatest in the least acid samples, whereas the total nitrogen content was greatest in the most acid samples.

There can be no question of any proportionality between time of heating and quantity of ammonia or total nitrogen. But it will easily be seen from the figures given, that an extrapolation would give at any rate very low values for the quantities formed in 0 hours' heating. In other words, the results obtained may easily be explained on the supposition that the actual denaturation does not cause any formation either of ammonia or of other nitrogenous substances, but that the decomposition in question takes place by the action of the hot water on the denaturated protein. The decomposition will therefore be the greater the longer the heating lasts, and the more of the denaturated protein there is in the solution. That is to say, the more the concentration of hydrogen ions diverges from the isoelectric reaction of egg albumin.

These experiments, however, do not give any decisive proof

<sup>1)</sup> Coagulation incomplete.



that no decomposition takes place in the process of denaturation itself. And we have therefore approached the problem from another aspect.

c) A solution of egg albumin entirely similar to that above described, was coagulated by means of 93% alcohol, which was added to the egg albumin solution a little at a time, with thorough shaking. The samples with alcohol were then left to stand for different periods, as will be seen from Table 3, where the results of this series of experiments are shown.

TABLE 3.

50 cc. egg-albumin solution, containing 1.050 g. egg albumin-N, used for each experiment: (pH = 4.7—4.8).

Experiment no.	Coagulation with alcohol				Heating of precipitate in boiling water bath		
	Quantity of alcohol used cc.	Standing for hours	<sup>100</sup> / <sub>250</sub> of filtrate contained		Time of heating hours	<sup>100</sup> / <sub>500</sub> of filtrate contained	
			Ammonia N in mg.	Total N in mg.		Ammonia N in mg.	Total N in mg.
Test I . . . .	50	—	0.04	0.07	—	0.01	0.04
Test II . . . .	100	—	0.04	0.07	—	0.01	0.04
1 . . . . .	50	1/2	0.06	0.09	1	0.05	0.14
2 . . . . .	50	2	0.04	0.08	1	0.04	0.10
3 . . . . .	50	5	0.04	0.06	1	0.05	0.17
4 . . . . .	50	32	0.05	0.06	4	0.13	0.24
5 . . . . .	50	72	0.05	0.06	4	0.13	0.31
6 . . . . .	25	32	0.04	0.06	18	0.29	0.60
7 . . . . .	100	32	0.04	0.07	28	0.43	0.70

After this standing, water was added to make a total volume of 300 c. c. After thorough stirring, repeated and careful shaking, and standing till next day, the precipitate was filtered off, and washed several times with cold water.

The filtrate and the washing water were then, after adding a little dilute hydrochloric acid, concentrated in a water bath to a small volume, in order to remove the alcohol, and the remainder then diluted to 250 cc, of which 100 cc was used for ammonia determination, and 100 cc for determination of the total nitrogen content.

The washed precipitates were transferred to conical flasks and heated with 300 cc of water on a boiling water bath for different lengths of time, as shown in Table 3. After being filtered and washed, the content of ammonia-N and total-N in the filtrates was determined.

Simultaneously with the experiments proper, two blank experiments (Tests I and II) were made without egg albumin solution, in order to determine the content of ammonia and total nitrogen in the water and reagents used.

Now the experimental error in an ammonia determination after Nessler can hardly be taken as less than 0.01—0.02 mg N. And in a Kjeldahl-Nessler determination, it will perhaps hardly be as low as this. We think, then, that the experimental results in the fourth and fifth vertical columns of Table 3 clearly show that during the coagulation of egg albumin with alcohol, neither ammonia nor other nitrogenous substances are formed — at any rate, not in such quantity as could be determined by the method here employed. Taking the smallest quantity of nitrogen demonstrable by this method as 0.02 mg, this would give a value for the whole filtrate answering to  $0.02 \cdot 250/100 = 0.05$  mg. or, in proportion to the total quantity of egg albumin nitrogen used in each experiment, (that is, 1.050 g.) about 1 : 20000.

We have shown in a previous work that the egg albumin molecule hardly contains more than abt. 380 atoms of nitrogen. Consequently, a simple calculation shows that the amount of nitrogen separated, if any, in our experiments, is at any rate less than the equivalent of one atom of nitrogen pr. 50 molecules of egg albumin.

We think then, that these experiments warrant the conclusion that during the denaturation of egg albumin with alcohol, there is no formation of ammonia or other nitrogenous substances.

From the three last vertical columns of Table 3 it will be seen that as soon as the denaturated egg albumin is heated with water, decomposition sets in, and on continued heating, the quantity both of ammonia and total nitrogen in the solution increases. This is entirely in agreement with the results of the previous series of experiments.

Under these circumstances then, it seems to us most natural

to assume that the actual denaturation itself by heating is, like the denaturation with alcohol, not accompanied by any decomposition, but that the decomposition is a secondary process, whereby the egg albumin during and after denaturation is decomposed by the action of the hot water.

d) In the case of egg albumin, the decomposition which can be supposed to take place on ordinary coagulation, as for quantitative determinations of egg albumin, will hardly be perceptible. If we take, for instance, experiment Nr. 3 in Table 3, we find that after the precipitate has been boiled for one hour, the amount of total nitrogen separated is only  $5 \cdot 0.17$  mg. in all, or hardly 1 o/oo of the whole amount of nitrogen present.

In the case of other proteins, however, as for instance serum albumin, and serum globulin, this source of error is not without importance, and analytical determinations of these substances by heat coagulation must therefore be corrected for the error as far as concerns determinations not of comparative, but of absolute value. It would take too long to describe in detail the various test experiments of this sort which we have made at one time and another. I will content myself with noting the principles on which the experiments were arranged, giving a single example by way of illustration.

When a coagulable protein, under optimal coagulation conditions, is not completely precipitated by heat coagulation, this may be due to the following causes:

*a)* That the denaturated protein is not absolutely insoluble in water, or in the salt solution in which the coagulation has taken place, or at any rate, that the precipitation is not quantitatively absolute,

*β)* That the heating, as we have just learned, causes a decomposition, accompanied by the formation of nitrogenous substances soluble in water and in salt solutions.

With regard to the source of error noted under *a)* we have presumed that even though the denaturated protein did not precipitate completely from the solution in which coagulation takes place, the precipitate once deposited will in any case not dissolve again on subsequent washing with hot water. This assumption contains no essential error in itself, and permits us to regard the correction originating from the source of error here

noted —  $x$  — as constant for the same protein, provided the coagulation is always effected in the same volume, without regard to the absolute quantity of protein. In our experiments, the volume was generally 100 cc.

And with regard to the source of error noted under  $\beta$ ) we have assumed — though this is only approximately correct — that the decomposition of the protein is a process which takes place uniformly at a constant temperature, so that the amount of "soluble nitrogen" will be directly proportional both to the time of heating and to the quantity of protein nitrogen present. If we call this last, expressed in milligrams,  $p$ , and the period of heating in hours,  $t$ , using  $y$  to denote quantity of "soluble nitrogen", in milligrams, formed per milligram protein nitrogen by one hour's heating, than the correction for this source of error will be  $p \cdot t \cdot y$ .

The total correction,  $n$ , will then be

$$n = x + p \cdot t \cdot y,$$

where the values of  $x$  and  $y$  must be determined by special test experiments with solutions of the protein in question, the amount of "coagulable nitrogen" and the amount of "non-coagulable nitrogen" being determined under different conditions of coagulation.

In these test experiments, it must be borne in mind that the protein solution used may possibly, even before the heating, already contain some non-coagulable nitrogenous substances. Such substances are extremely easily formed from the coagulable proteins, through the action of bacteria, or merely through leaving the solution to stand at ordinary or even at rather low temperature, as for instance, in lengthy dialysis. The error originating from this source is of course proportional to the quantity of protein solution used in the experiment, but independent of the time of coagulation and of the volume during coagulation. It can therefore be expressed as  $p \cdot z$ , where  $z$  denotes the quantity of "non-coagulable nitrogen" expressed in milligrammes, contained in the protein solution used, per milligram of "coagulable nitrogen". In the test experiments therefore, the quantity of "non-coagulable nitrogen" found,  $m$ , must be expressed as follows:

$$m = x + p \cdot t \cdot y + p \cdot z.$$

And by means of the values found, or given, for  $m$ ,  $p$  and  $t$ , the values for  $x$ ,  $y$  and  $z$  can be calculated.

I will not go into the question of analytical methods, but merely mention that the test experiments fall mainly into two series. In the one series, the time of heating,  $t$ , is varied, all other factors remaining constant; in the other, the quantity of protein,  $p$ , is varied, the remaining factors being kept constant. The first series enables us to determine  $y$  and  $x + p \cdot z$ , whereas the second gives us  $x$  and  $z$ .

In the case of the first series, the values found for  $m$  were used as ordinates and for  $t$  as abscissæ. Through the points thus found, or as nearly as possible, a straight line was drawn, answering to the equation:

$$m = (p \cdot y) t + (x + p \cdot z).$$

The tangent to the angle of inclination is therefore equal to  $p \cdot y$ , from which  $y$  can be calculated, while  $x + p \cdot z$  is found as the value of  $m$  for  $t = 0$ .

In the second series, the values found for  $m$  were used as ordinates, and those for  $p$  as abscissæ; through the points thus found was drawn, as far as possible, a straight line, answering to the equation:

$$m = (z + y \cdot t) \cdot p + x.$$

The tangent to the angle of inclination is thus  $z + y \cdot t$ , from which  $z$  can be found, since  $y$  is known, while  $x$  is found, either as the value of  $m$  for  $p = 0$  or from the value for  $x + p \cdot z$  found in the first series.

Table 4 and Figure 1 show the results of two such series of experiments with an easily soluble fraction of serum albumin.

It will be seen from Table 4, series I, that the quantity of "coagulable" N found decreases, and that of "non coagulable" N increases with increasing time of heating. It will further be seen, from series II, that the quantity of "non coagulable" N, though not proportional to the quantity of protein, nevertheless increases with the same.

Figure 1 shows the results of the experiments in graphical form, and it will at once be seen that the accuracy of these determinations is not quite so high as could be wished, the error in some cases evidently amounting to 0.05 mg. "non coagulable" N. We do not think, however, that a higher degree of

TABLE 4.

## Test experiments with an easily soluble fraction of serum albumin.

$$\text{Correction term: } m = x + p. t. y + p. z \begin{cases} x = 0.11 \\ y = 0.0076 \text{ (t. in hours)} \\ z = 0.0009 \end{cases}$$

Series of Experiments	Time of heating	Total content of serum albumin N	Found			Calculated correction $m = x + p. t. y + p. z$			
			Coagulable N	Non-coagulable N	Total content of serum N	x	p.t.y.	p. z.	m
	minutes	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
I Influence of time of heating	10	25.50	25.31	0.22	25.53	0.11	0.03	0.02	0.16
	15	do.	25.29	0.20	25.49	0.11	0.05	0.02	0.18
	20	do.	25.28	0.24	25.52	0.11	0.06	0.02	0.19
	25	do.	25.17	0.23	25.40	0.11	0.08	0.02	0.21
	30	do.	25.20	0.20	25.40	0.11	0.10	0.02	0.23
	45	do.	25.10	0.30	25.40	0.11	0.15	0.02	0.28
	60	do.	25.09	0.37	25.46	0.11	0.19	0.02	0.32
	120	do.	24.89	0.55	25.44	0.11	0.39	0.02	0.52
II Influence of quantity of protein	45	5.10	4.88	0.13	5.01	0.11	0.03	0.00	0.14
	do.	10.20	9.84	0.12	9.96	0.11	0.06	0.01	0.18
	do.	15.30	14.94	0.18	15.12	0.11	0.09	0.01	0.21
	do.	20.40	20.01	0.21	20.22	0.11	0.12	0.02	0.25
	do.	25.50	25.10	0.30	25.40	0.11	0.15	0.02	0.28
	do.	30.60	30.19	0.30	30.49	0.11	0.17	0.03	0.31
	do.	51.00	—	0.44	—	0.11	0.29	0.05	0.45
	do.	102.00	—	0.76	—	0.11	0.58	0.09	0.78

accuracy could be obtained without great inconvenience. And it is a matter of no great difficulty, on the basis of the experimental results, to draw the two straight lines by means of which the calculation of x, y and z can be made as above described.

The calculation gives, in the present instance,  $x = 0.11$ ,  $y = 0.0076$  (for t in hours) and  $z = 0.0009$ . And it will be seen from Table 4, on comparing the fifth and last vertical columns, that the correction calculated by this formula agrees well with the quantities of "non coagulable" N found in the experiments.

The magnitude of the correction in proportion to the total quantity of protein nitrogen is, as will be seen from the table, somewhat different according to the time of heating and the concentration of the protein. But it may easily reach a value

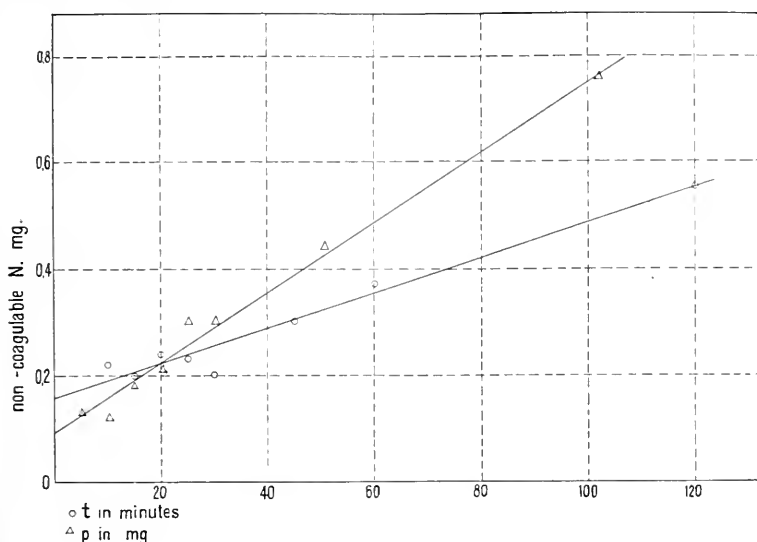


Fig. 1.

of 1%, and at low concentrations, the correction is considerably higher.

In accurate determinations of the content of "non coagulable" N in a solution beside its "coagulable" N, as for instance, in determining the so-called "non protein nitrogen" of blood by the heat coagulation method, this source of error must therefore be taken into consideration.

## B. Does the Protein give off or take up water during the Process of Denaturation?

a) To the second question before us it is far more difficult to give a definite and final answer. Direct determination of the water content of the protein before and after denaturation cannot be carried out, as the evaporation of a protein solution, or the dessication of crystallised, precipitated or denaturated proteins usually results in a complete dehydration, leaving it impossible to determine whether any of the water given off — and if so, how much, — was an original constituent of the protein.

In such cases, we are obliged to be content with indirect methods. And I will in this connection refer to the so-called "Proportionality Method" used at the Carlsberg Laboratory. We have applied this method with advantage in determinations of the water content of crystallised egg albumin<sup>1)</sup> and also in the study of the denaturation process.

I cannot here go far into details regarding the principle and application of the proportionality method. I will merely point out that we can, by analysis both of a precipitate with its surrounding mother liquor, and of the mother liquor itself without precipitate, gain certain information as to the composition of the precipitate. That is, provided the mother liquor adhering to and surrounding the precipitate is of the same composition as the mother liquor filtered off or otherwise removed for analysis.

We have, say, an albumin precipitate which has been precipitated or crystallised out by ammonium sulphate. The respective weights may be denoted as follows:

Ammonia nitrogen	per 100 g. filtrate (mother liquor) . . . . .	$a_f$
do.	per 100 g. precipitate with adherent mother liquor . . . . .	$a_b$
Protein nitrogen	per 100 g. filtrate (mother liquor) . . . . .	$p_f$
do.	per 100 g. precipitate with adherent mother liquor . . . . .	$p_b$

We can then show, that the factor,  $x$ , by which the weight of protein nitrogen in the precipitate must be multiplied to give the weight of the hydrated protein, may be expressed as follows:

$$x = \frac{100 (a_f \div a_b)}{a_f \cdot p_b \div a_b \cdot p_f}$$

By means of analyses of this sort we have succeeded in showing that  $x$  for crystallised egg albumin has a value independent of the conditions of crystallisation, 7.86, while the factor by which the weight of protein nitrogen must be multiplied to give the weight of non-hydrated egg albumin is 6.4. This means, as a simple calculation will show, that crystallised egg albumin contains 0.22 g of water for every gram of water-free egg albumin.

The formula here used for  $x$  holds good only in cases

<sup>1)</sup> S. P. L. Sørensen and Margrethe Høyrup: Comptes-rendus du Lab. Carlsberg **12**, 164 (1917).



where the protein does indeed contain water, but has no bound ammonia or ammonium sulphate. In the great majority of cases, however, we have to reckon with the fact that the protein, both in solution and as a precipitate, has bound some sulphate of ammonia (or other electrolytes present). The formula then becomes more complicated. If we take  $z$  and  $y$  respectively for the factors by which the weight of protein nitrogen must be multiplied to give the weight of protein + water + ammonium sulphate (factor  $z$ ) in the one case, and the weight of ammonium sulphate therein (factor  $y$ ) in the other, we can apply the following formula:

$$z = x + y = r + s \cdot y,$$

$$\text{where } r = \frac{100 (a_f \div a_b)}{a_f \cdot p_b \div a_b \cdot p_f} \text{ and } s = \frac{100 (p_b \div p_f)}{4.7163 (a_f \cdot p_b \div a_b \cdot p_f)}$$

Now experiments shows, as was also to be expected, that  $y$  is always quite small in comparison with  $x$ , but  $y$  is usually not equal to 0 and only in this case is  $z = x = r$ . On the other hand,  $s \cdot y$  need not be small in comparison with  $r$ ;  $s \cdot y$  will naturally be of higher importance the greater  $y$  is, or in other words, the greater the amount of ammonium sulphate bound by the protein is and the greater  $s$  is. This again, as examination of the formula for  $s$  will show, is equivalent to saying the lower the concentration of ammonium sulphate. The value found by experiment for  $r$  will thus only be equal to  $z$  for  $y = 0$ , and for the rest, it will be found to be less the greater  $s \cdot y$  is, or again the smaller the ammonium sulphate content in the mother liquor ( $a$ ) and the greater  $y$  is. Under otherwise uniform conditions,  $r$  thus alters with  $a_f$ , and the more strongly the less  $a_f$  is, so that we can, from these alterations in the magnitude of  $r$ , form an idea as to the magnitude of  $y$ . It would take too long to go further into the considerations on which this approximate determination of  $y$  is based; it must suffice here to mention the variation of  $r$  for varying values of the concentration of ammonium sulphate.

The curves in Fig. 2 show the results of some series of experiments of various kinds which we must now consider more closely. All the curves express the relation between the value of  $r$  and concentration of ammonium sulphate in the mother liquor,  $r$  being used as ordinate, while the quantity of ammonium sulphate,  $S$ , in the mother liquor per 100 g. water is used as abs-

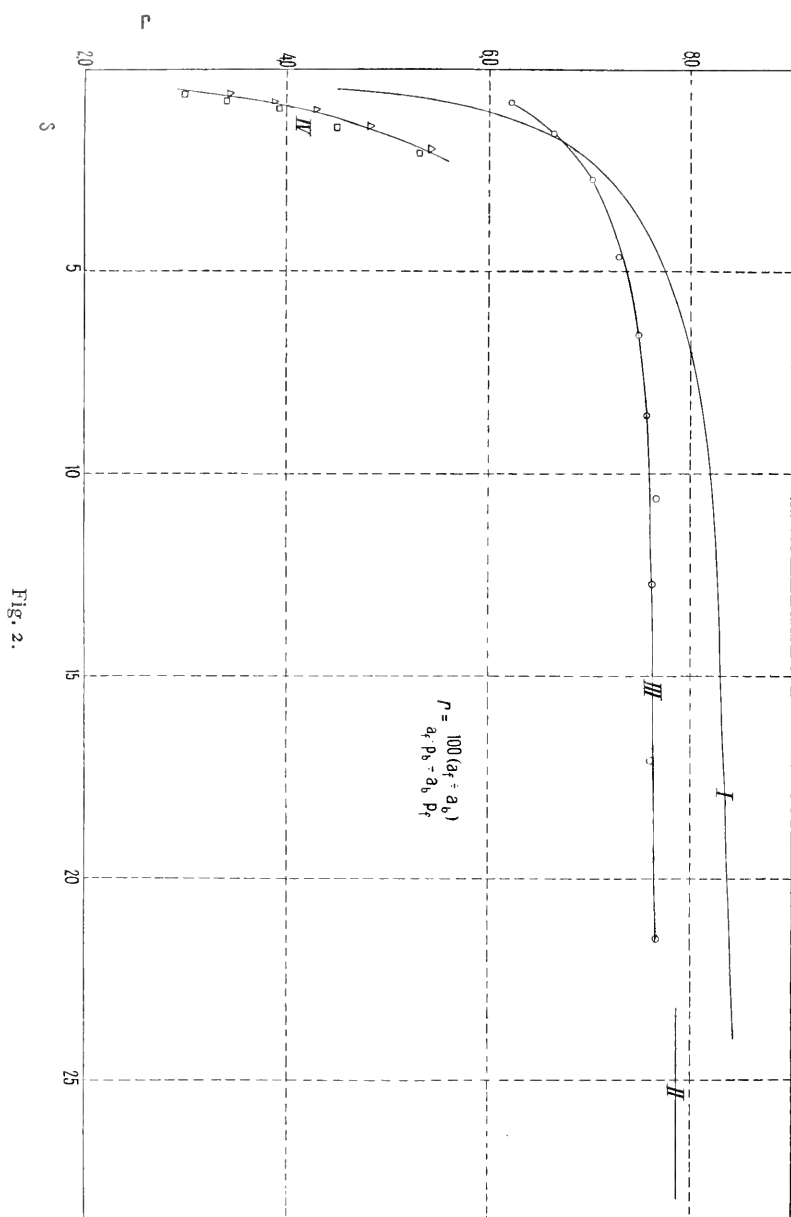


Fig. 2.

cissæ. The concentration of hydrogen ions in all experiments answers to  $p_H = 4.7 - 4.8$ .

b) Curve I is borrowed from a previous work<sup>1)</sup> and expresses

<sup>1)</sup> S. P. L. Sørensen: Comptes-rendus du Lab. Carlsberg **12**, 362 (1917).

the relation between  $r$  and  $S$  for ammonium-sulphate-containing aqueous solutions of egg albumin with  $p_H = \text{abt. } 4.8$ . In such solutions, the disperse phase, that is, the hydrated protein, answers to what we have above termed the precipitate, whereas the dispersion medium, the aqueous ammonium sulphate solution surrounding the disperse phase, answers to the adherent mother liquor. If an egg albumin solution of this sort be placed as "inside solution" in a collodium tube, which again is placed in an "outside solution" of the same composition as the dispersion medium in the "inside solution", then we have a system to which we can apply the mode of consideration noted above, and the corresponding formulæ, as long as care is taken to maintain diffusion equilibrium and osmotic equilibrium between the inside and outside solutions. It will be seen from curve I that  $r$  at high concentrations of ammonium sulphate is very nearly constant (abt. 8.4); with a decrease in  $S$ , the value of  $r$  falls, quite slowly at first, and then more and more rapidly. The reason why  $r$  is not found constant is, as already mentioned, that the egg albumin in a solution containing ammonium sulphate has combined with some ammonium sulphate, with the result that  $a_b$  is found too high, and  $r$ , calculated from the formula

$$r = \frac{100(a_f - a_b)}{a_f \cdot p_b - a_b \cdot p_f}$$

therefore too low.

We can see from the shape of the curve that  $y$  must have a value rising with  $S$  from 0.01 to 0.05, and the maximal value of  $z$  can therefore be calculated as abt. 8.7, while  $x$  will be abt.  $8.7 - 0.05$ , or abt. 8.65. And as the factor by which the weight of protein nitrogen must be multiplied to give the weight of water-free protein is 6.40, we find that the egg albumin in an aqueous solution under these conditions will contain, for each gram of water-free protein,  $\frac{8.65 - 6.40}{6.40}$  or 0.35 g of water.

c) Before describing our denaturation experiments, I must just mention that Curve II, which appears as a straight line answering to  $r = 7.86$ , represents the crystallised egg albumin, whose water content we have, as already mentioned, found constant, independent of the concentration of ammonium sulphate. This curve comprises only the high concentrations of ammonium sulphate, as egg albumin, of course, does not crystallise out at

lower concentrations.  $r = x = z = 7.86$  answers to a water content of 0.22 g. water per gram of water-free egg albumin, so that even on crystallisation of the egg albumin, a certain amount of water is already given off.

d) Curve III shows the results of a heat coagulation experiment, in which a series of solutions, all at the same concentration

TABLE 5.

Experiment no.	100 g. filtrate contained Ammonia-N in g.	Filtrate's content of Ammonium sulphate in g. pr. 100 g. water	r (found)			Mean of r
	$a_f$	S				r
1	0.1729	0.822	6.23 <sub>9</sub>			6.24
2	0.3272	1.567	6.58 <sub>0</sub>	6.69 <sub>5</sub>	6.63 <sub>5</sub>	6.64
3	0.5595	2.710	6.96 <sub>1</sub>	6.99 <sub>1</sub>	7.08 <sub>1</sub>	7.01
4	0.9393	4.635	7.20 <sub>3</sub>	7.29 <sub>7</sub>	7.33 <sub>5</sub>	7.28
5	1.3074	6.571	7.54 <sub>0</sub>	7.41 <sub>1</sub>	7.48 <sub>0</sub>	7.48
6	1.6722	8.562	7.64 <sub>2</sub>	7.53 <sub>0</sub>	7.47 <sub>5</sub>	7.55
7	2.0369	10.628	7.65 <sub>6</sub>	7.69 <sub>3</sub>	7.66 <sub>3</sub>	7.67
8	2.3882	12.693	7.67 <sub>3</sub>	7.58 <sub>5</sub>	7.55 <sub>6</sub>	7.61
9	3.0957	17.096	7.59 <sub>6</sub>			7.60
10	3.7563	21.530	7.61 <sub>2</sub>	7.75 <sub>5</sub>	7.59 <sub>3</sub>	7.65

of hydrogen ions, answering to  $p_H = 4.7$ , and all containing the same amount of egg albumin, but with different contents of ammonium sulphate, were coagulated by heating for one hour in a boiling water bath with good and repeated shaking. After standing for a couple of days, they were filtered, and samples both of the filtrate and of the precipitate with adherent mother liquor were weighed. In the former, the amount of ammonium sulphate was determined, in the latter, both the amount of Ammonia-N and of egg albumin nitrogen, after which  $r$  was calculated according to the formula given above. The results of the experiments are tabulated in Table 5 and shown graphically in Curve III (Fig. 2).

It will be seen that the results of these experiments can easily and naturally be arranged in a curve, and there is hardly any doubt, therefore, that this curve can be used to elucidate the question at issue. At high concentrations of ammonium

sulphate,  $r$  is very nearly constant (7.6—7.7) with decreasing  $S$ , the value of  $r$  falls, quite slowly at first, and then faster and faster. The value of  $r$  for the coagulated egg albumin is, as will be seen from the figure, still less than for the crystallised egg albumin, and far less than for the dissolved egg albumin. This may be due to the fact that the denaturated egg albumin contains less water than the dissolved. But it can also be explained, if we may assume that the denaturated egg albumin has bound more ammonium sulphate than the dissolved at the same concentration of ammonium sulphate. However, a closer examination of the formulæ given above shows that the quantity of ammonium sulphate bound per gram-equivalent of egg albumin nitrogen must be the greater, the steeper the course of the curve. It is also very plainly evident from the figure, that Curve III is far less steep than Curve I. The experiment shows, then, that the denaturated egg albumin, under otherwise equal conditions, has bound less ammonium sulphate, and contains considerably less water than the dissolved. On the other hand, at a fairly high concentration of ammonium sulphate,  $r$  has a far higher value than 6.4, from which we may conclude that only a portion of the water was lost by the denaturation process.

e) Curve IV, Figure 2, shows some of the results from a series of denaturation experiments with egg albumin by means of alcohol.

This series comprises four groups of experiments at different concentrations of hydrogen ions, answering to  $p_H$  approximately equal to = 4.45, 4.73, 4.82 and 5.03 respectively. For each individual experiment, the same quantity of egg albumin was used (answering to 0.378 g. egg albumin nitrogen), but the concentration of ammonium sulphate varied from one experiment to another within each group. Naturally, in these experiments, the ammonium sulphate concentration had to be comparatively low, as otherwise, the addition of alcohol would precipitate ammonium sulphate.

The experiments were carried out simply as follows: To 30 cc of the ammonium-sulphate-containing egg albumin solution, 90 cc of 90% alcohol was added gradually, shaking well the while. After standing for at least 24 hours, 120 cc of water was added, and the whole then left to stand for two or three days, with thorough shaking from time to time, to give complete equi-

TABLE 6.

Experiment no.	100 g. filtrate contained Ammonia-N in g. $a_f$	Filtrate's content of Ammonium sulphate in g. pr. 100 g. water S	r (found)	Concentration of hydrogen ions in the solution before denaturation $P_H$
1	0.1267	0.6012	5.39	abt. 4.45
2	0.1652	0.7852	5.41	do.
3	0.2066	0.9840	5.62	do.
4	0.2910	1.3915	5.94	do.
5	0.4098	1.9708	6.15	do.
6	0.1250	0.5930	3.43	abt. 4.73
7	0.1673	0.7953	3.87	do.
8	0.2079	0.9902	4.29	do.
9	0.2868	1.3711	4.83	do.
10	0.4128	1.9856	5.42	do.
11	0.1278	0.6064	2.99	abt. 4.82
12	0.1637	0.7781	3.41	do.
13	0.2054	0.9782	3.94	do.
14	0.2905	1.3891	4.50	do.
15	0.4219	2.0302	5.31	do.
16	0.1264	0.5997	0.88	abt. 5.03
17	0.1665	0.7915	1.84	do.
18	0.2093	0.9969	2.40	do.
19	0.2884	1.3790	3.59	do.
20	0.4123	1.9831	4.13	do.

brium between the precipitate and the surrounding mother liquor. It was then filtered, and samples of the filtrate, as well as of the precipitate with adherent mother liquor, were weighed. In the former, the quantity of ammonia nitrogen was determined, in the latter, both the quantity of ammonia-N and egg albumin-N, after which  $r$  was calculated according to the usual formula. The results of these experiments are shown in Table 6, and graphically in Fig. 3.

Both the table and the figure show that the value of  $r$  alters both with the concentration of hydrogen ions and with that of ammonium sulphate. And the alteration proceeds, qualitatively speaking, as we should expect. We must bear in mind the fact

that the alteration in the value of  $r$  depends on a difference in the amount of ammonia bound by the coagulated egg albumin in the different experiments, which must be assumed to be least in the most acid mixtures. And we must also remember that one and the same quantity of ammonia bound will, in the calculation of  $r$ , be more important as the total quantity of ammonium sul-

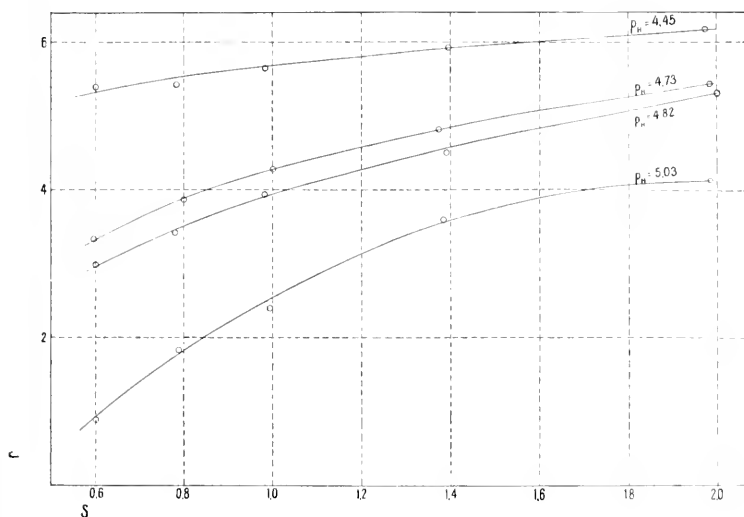


Fig. 3.

phate present decreases. These points considered, it is easy to understand the course of the curves. At the same concentration of ammonium sulphate,  $r$  must appear greater and greater the higher the concentration of hydrogen ions, and at the same concentration of hydrogen ions,  $r$  will increase with the concentration of ammonium sulphate.

It will be seen from Fig. 3, which is drawn on a larger scale, that the results of the experiments are easily and naturally arranged in the four curves answering each to one of the groups of experiments.

In Fig. 2, Curve IV shows the two middle groups of this series, answering to  $p_H = 4.7 - 4.8$ . Only one curve has been drawn, representing both groups, but it will be seen from the figure that all the points answering to the one group lie on one side of the curve, and all those of the other group on the other side. The curve shows a far steeper course than those in Fig. 3,

because the scale, in the case of the abscissæ, is much smaller in Fig. 2 than in Fig. 3.

On comparing Curve IV with the other curves in Fig. 2, it will be seen that the same ammonium sulphate concentration gives  $r$  still lower for the egg albumin denaturated with alcohol than for that denaturated by heating. The curve, however, is steeper than Curve III but less steep than Curve I, which suggests that the egg albumin denaturated by alcohol has, under otherwise equal conditions, bound more ammonium sulphate than the heated sample, but less than the dissolved. Curve IV however, does not embrace such high concentrations of ammonium sulphate that we can draw absolutely certain conclusions from its course, but a closer study of the results of the experiments leaves very little doubt that the alcohol denaturation also has caused a considerable giving off of water.

f) We have further endeavoured in yet another way to get nearer to a solution of the problem under consideration. The difficulty of interpreting the results of the above noted experiments lies first and foremost in the fact that the substance, ammonia or ammonium sulphate, whose determination in "filtrate" or "precipitate with adherent mother liquor" gives the data necessary for calculating  $r$ , combines with — or if you will, is adsorbed by — the egg albumin. It seemed therefore natural to attempt a determination of  $r$  by using another substance than ammonium sulphate. And as it might be assumed that a non-electrolyte would be far less liable to adsorption by the egg albumin than any of the electrolytes, we have carried out a series of experiments with glucose as the "common analytical substance" in "filtrate" and "precipitate with adherent mother liquor". Hr. H. Jessen-Hansen has kindly undertaken all the sugar determinations for these experiments.

The method of procedure here was simply as follows: An egg albumin solution containing ammonium sulphate was denaturated by alcohol, and the precipitate, after addition of water and thorough shaking, filtered off and washed with cold water, until practically all the alcohol and ammonium sulphate were washed away. The moist precipitate was then distributed between three flasks, and 200 cc of a glucose solution at different concentrations poured on. After standing for 3 days with thorough and repeated shaking, to ensure completely uniform distribution



of the sugar, it was filtered, and samples both of the filtrate and of the precipitate with adherent mother liquor were weighed. In the former the quantity of sugar was determined, and in the latter both quantity of sugar and of egg albumin nitrogen, after which  $r$  was calculated according to the proportionality method, using the formula:

$$r = \frac{100 (S_f - S_b)}{S_f \cdot p_b - S_b \cdot p_f}$$

I cannot here go into the methods of analysis, but merely give a survey of the results in Table 7.

TABLE 7.

Experiment no.	100 g. filtrate contained glucose in g. $S_f$	100 g. precipitate with adherent sugar solution contained		$r$	Mean of $r$
		glucose in g. $S_b$	Egg albumin nitrogen in g. $p_b$		
1 a . . . . .	21.580	19.751	1.1742	7.23	7.49
1 b . . . . .	do.	19.663	1.1827	7.49	
1 c . . . . .	do.	19.572	1.2021	7.74	
2 a . . . . .	10.637	9.616	1.2711	7.49	7.49
2 b . . . . .	do.	9.629	1.2570	7.55	
2 c . . . . .	do.	9.615	1.2985	7.42	
3 a . . . . .	5.125	4.533	1.3386	8.64	8.13
3 b . . . . .	do.	4.585	1.3568	7.74	
3 c . . . . .	do.	4.581	1.3253	8.00	

It will be seen from Table 7 that the agreement between the individual experiments is not as good as might be wished. This applies more especially to those experiments where the sugar concentration is low, and the difference between  $S_f$  and  $S_b$  therefore inexact. It appears, however, plainly enough from the experiments that there is no question of any demonstrable adsorption of sugar by the egg albumin, as  $r$  in that case would decrease with decreasing sugar concentration. We must presume, then, that the value found for  $r$ , within the limits of error, gives the factor by which the weight of egg albumin nitrogen must be multiplied to give the weight of denaturated egg albumin

with its content of water. Taking  $r$  as 7.5, without regard to the experiments with weak sugar solution, this means that the denaturated egg albumin contained

$$\frac{7.5 - 6.4}{6.4} = 0.17 \text{ g water pr. gram water-free egg albumin.}$$

The main result of the experiments dealt with in this section can then, we think, be expressed as follows:

In the denaturation of egg albumin, both by alcohol and by heating, the egg albumin gives off water, but it has not proved possible to determine how far the giving off of water extends, or whether it is in all cases of the same extent. It must be considered as proved, however, that the denaturated egg albumin still always contains some water.

In conclusion, I should like to describe to you a simple experiment which can very well be used for lecture purposes, and gives a very clear view of the two processes of coagulation: the denaturation and the flocculation.

The experiment in question has already been described in a paper of mine in 1916, and the matter itself should, after the publication of Chick and Martin's works, which I have referred to above, be well known. But in the ordinary handbook literature there is rarely, if ever, any distinction made between denaturation and flocculation. And I have therefore considered it worth while bringing up this experiment again.

In order to get the work out on as clear and sharp lines as possible, we use for this experiment a purified protein solution, as for instance an egg albumin solution purified by repeated recrystallisation and subsequent dialysis. The solution may be as nearly as possible free from salt, as any quantity of salt, however small, tends to accelerate the flocculation.

In each of the 12 test tubes (Fig. 4a) is placed 5 cc of such a pure egg albumin solution, containing 40—50 mg albumin nitrogen, and 15 cc of (water + hydrochloric acid), so distributed that the tube on the extreme left has 15 cc water without hydrochloric acid, the quantity of this last increasing untill we reach the test tube on the extreme right, which has 15 cc n/100 hydrochloric acid.

After heating for 15 minutes in a water bath at a temperature of 60—65°, the samples will present the appearance shown in Fig. 4a. Nos. 1 and 2 will be perfectly clear; there is precipitate in Nos. 3 to 6, most in No. 4, whereas the rest have no precipitate, but only an opalescence, which decreases from left to right. It would thus seem as if there had only been coagulation in the middle samples, whose concentration of hydrogen ions lies near the isoelectric reaction of egg albumin. In reality, however, this is not the case. On filtration, we find that Nos. 1—6 filter easily, the others with difficulty. We then add at ordinary temperature a mixture of sodium acetate and acetic acid, which brings all the filtrates to the optimal concentration of hydrogen ions for flocculation, answering to  $p_H = 4.7 - 4.8$ , and we now get a precipitate in all the samples except Nos. 3 and 4, and most in the most acid samples, as shown in Fig. 4b.

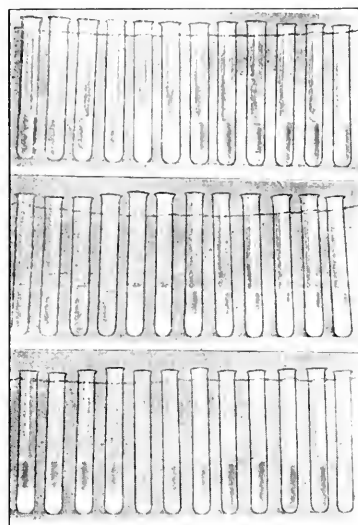


Fig. 4.

As all the samples have now the optimal concentration of hydrogen ions for flocculation, the entire amount of denaturated egg albumin will have been precipitated, and if we filter the samples and heat them anew, the egg albumin still remaining in the filtrates and not yet denaturated will thereby be denaturated and precipitated. Fig. 4c shows that this last heating gives precipitate only in Nos. 1—9 and a greater quantity of precipitate according as the liquid was less acid.

It appears, then, that the egg albumin in the most acid samples (the test tubes farthest to the right) was completely denaturated by the first heating, but no precipitation took place, because the concentration of hydrogen ions was far higher than the optimum for flocculation. On the other hand, the sample which yielded most precipitate on the first heating, No. 4, was by no means completely denaturated, since it again yields abundant precipitate on the second heating. But all what was

denaturated at the first heating has also been precipitated (see Fig. 4b) because this sample had the optimal concentration of hydrogen ions for flocculation.

Under otherwise equal conditions, then, the rate of denaturation will be higher, the higher the concentration of hydrogen ions in the solution, but the flocculation of the denaturated protein only takes place at isoelectric reaction, or near it. The addition of salt extends the limits for complete flocculation, giving a zone with isoelectric reaction as the optimal concentration of hydrogen ions.

I shall not have time today to go into the very important question as to the influence of the various electrolytes on denaturation, and on flocculation. It is a highly complicated question, and we are at present occupied with its investigation.

I am quite aware, then, as I mentioned at the beginning of my lecture, that this important question, the problem of proteins and their coagulation, is far from being solved by the investigations I have had the honour to put before you today. Nevertheless, I hope I have given you here some slight contribution towards a better understanding of what takes place.

*July 1924.*

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COMPTES-RENDUS

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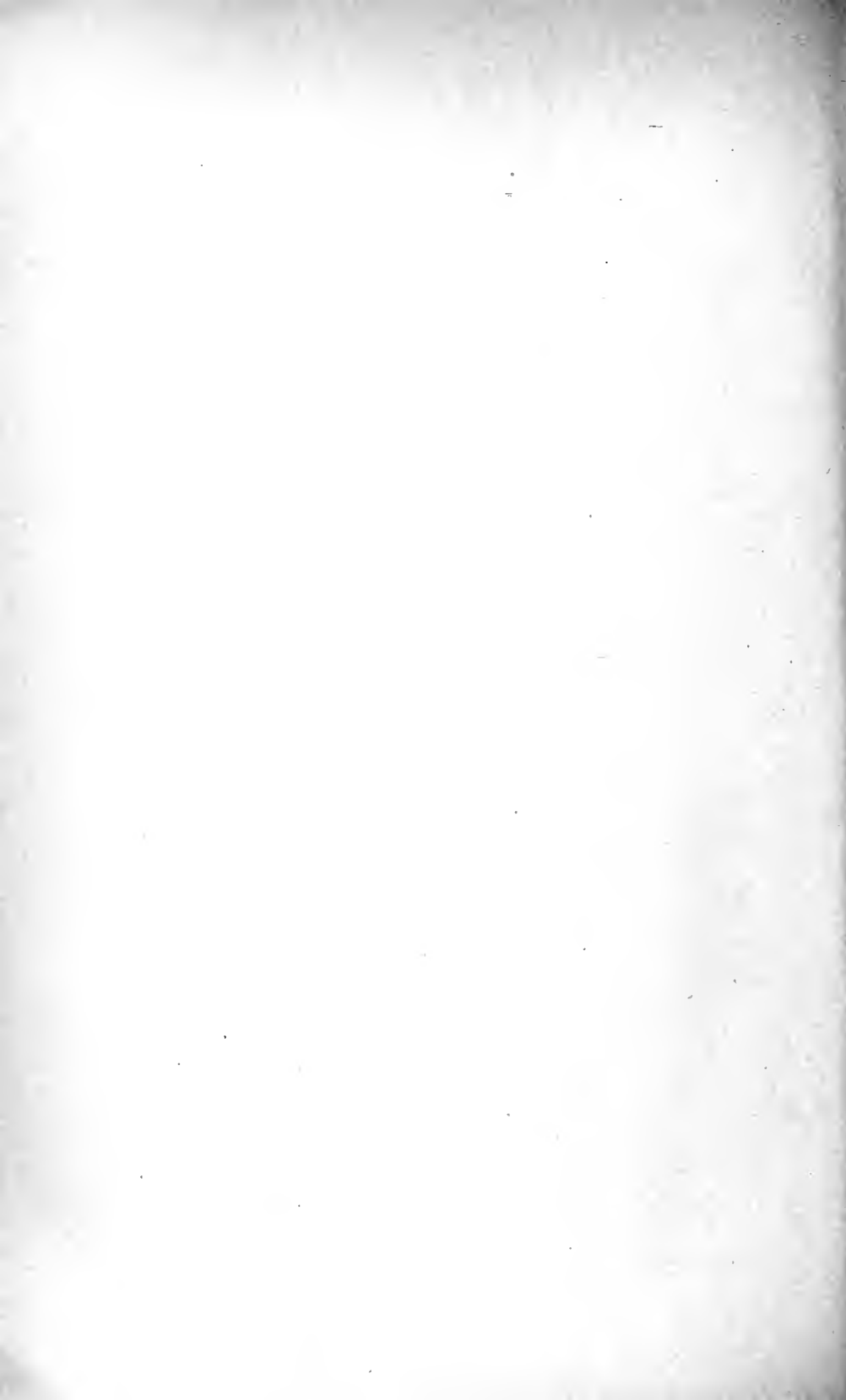
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# ON THE DETERMINATION OF SMALL QUANTITIES OF PHOSPHORUS IN PROTEINS.

BY

MARGRETHE SØRENSEN.

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In the investigations with proteins carried out during the past few years at Carlsberg Laboratory, it has been found desirable to have an exact method of determining small quantities of phosphorus in relatively large amounts of protein.

We endeavoured first of all to use a colorimetric method indicated by Richard D. Bell and Edward A. Doisy<sup>1)</sup>, afterwards revised by A. P. Briggs<sup>2)</sup> and F. S. Randles and Arthur Knudson<sup>3)</sup>. The method in question, which does not require any previous precipitation of the phosphate as ammonium phosphomolybdate, is a comparatively easy one, but the colour in the colorimetric measurement is far from permanent, and very susceptible to the influence of accidental external factors. Indeed, as far as our researches are concerned, the method only afforded an estimate as to the order of magnitude of the amount of phosphorus present. We endeavoured therefore, to bring about more uniform conditions in the production of the colour by precipitating any phosphorus present as ammonium phosphomolybdate, dissolving this in ammonia and then adding the reagents required for producing the colour. This renders the method much more complicated, and even then, the agreement between the analyses was not satisfactory.

We have therefore reverted to the method generally employed: precipitation with ammonium molybdate and titration.

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<sup>1)</sup> Journ. of Biolog. Chemistry **44**, 55 (1920).

<sup>2)</sup> Ibid. **53**, 13 (1922).

<sup>3)</sup> Ibid. **53**, 53 (1922).

The method was elaborated by A. Neumann<sup>1)</sup>, and further studied by J. P. Gregersen<sup>2)</sup>. On the basis of this, Richard Kuhn<sup>3)</sup> has worked out a micro-method, dissolving the phosphomolybdate in  $n/10$  sodium hydroxide and titrating with  $n/10$  acid.

O. Svanberg, K. Sjöberg and G. Zimmerlund<sup>4)</sup> have likewise worked out a micro-method, finding the quantity of phosphorus by determining the ammonia entering into the phosphomolybdate precipitate according to Bang's micro-Kjeldahl method.

None of these variations of the method proved altogether satisfactory in our case, where we had as a rule to determine very minute quantities of phosphorus (0.02 — 1 mg. P) in quite considerable quantities of organic matter (often more than 1 g of the protein), so that a micro-method can at any rate only be used for part of the process.

**The Method Employed.** We have found that the method described in the following, a combination of other known methods, gives the best results under the circumstances here in question.

The protein solution in question is dried up in long-necked Kjeldahl flasks, whereafter the digestion by 4 cc conc. sulphuric acid and 4 cc conc. nitric acid, takes place, heating cautiously at first, then subjecting to higher temperature. If, when the nitric acid has been boiled away, the liquid is not perfectly clear, and more than slightly yellowish in colour, then more nitric acid is cautiously added, but no more sulphuric acid, as test experiments have shown this quantity of sulphuric acid to be adequate under the given conditions of precipitation. When the digestion is completed, the whole is left to cool, after which 25 cc water and 10 cc saturated ammonium nitrate solution should be added. The samples are now heated in a boiling water bath and 25 cc of a 5 % ammonium molybdate solution are added. The heating in boiling water bath is continued, with repeated shaking, for 5 minutes. In order to make sure that all the ammonium phosphomolybdate has been precipitated, the samples are left to stand until next day, and then filtered through the Berzelius filter (No. 0,

<sup>1)</sup> Zeitschr. physiol. Chem. **37**, 115 (1920).

<sup>2)</sup> Ibid. **53**, 453 (1907).

<sup>3)</sup> Ibid. **129**, 64 (1923).

<sup>4)</sup> Arkiv för Kemi, Mineralogi och Geologi **8**, Nr. 10 (1921).

diameter 7 cm). Care should be taken to get as little as possible of the precipitate on the filter. The precipitate is washed four times with ice-cold water, and eight times with ice-cold 50 % alcohol. The deposit on the filter is dissolved by dropping on 5 cc 2*n* ammonia; the solution then runs down into the Kjeldahl flask, where the main quantity of the precipitate is found. The filter is washed by filling up three times with water. To the solution + washing water is added an accurately measured amount of *n*/10 sodium hydroxide, as much as would seem to be 3—5 cc more than answering to the molybdate precipitate, and all the ammonia is boiled away. (Boil for about an hour, when the volume will be reduced from abt. 60 cc to 10—15 cc. Three or four small pieces of pumice stone may be added to make the liquid boil steadily.) Surplus sodium hydroxide is titrated with *n*/10 sulphuric acid, 1—2 cc *n*/10 sulphuric acid further added, and the carbonic acid boiled away (boil for 8—10 minutes). After cooling, the sulphuric acid is titrated with *n*/10 sodium hydroxide, and an excess of obt. 0.5 cc *n*/10 sodium hydroxide further added, as the pumice stone may keep back a little of the sulphuric acid. After standing for abt. half an hour, the titration is completed. Two or three blank experiments are carried out through all the operations, and used as a test, the quantity of sodium hydroxide thus expended being subtracted from the analyses.

The Factor. Supposing the composition of the ammonium phosphomolybdate to be such that for each atom of phosphorus, on titration, 28 equivalents of base will be used, then the factor by which the number of cc *n*/10 sodium hydroxide used must be multiplied to give the amount of phosphorus in milligrammes will be:

$$\frac{31.04}{28 \times 10} = 0.111.$$

This calculated factor will probably always give too low results, as a very small quantity of the yellow precipitate will be dissolved in the washing. We have therefore thought it better to use a factor determined by analysis, made in the manner above described, of a solution with known content of phosphoric acid.

0.1169 g primary potassium phosphate (Kahlbaum's »Zu Enzymstudien nach Sorensen«) was dissolved in water to 250 cc.

Weight of the solution: 249.50 g. From this, 5 samples of 10 cc were taken (weight of 10 cc: 9.927 g).

Quantity of n/10 sodium hydroxide used:

I —	9.45 cc
II —	9.45 "
III —	9.49 "
IV —	9.49 "
V —	9.46 "
<hr/>	
	9.47 cc

The factor will then be:

$$\frac{0.1169 \times 9.927 \times 1000}{249.50 \times 9.47} \times \frac{31.04}{136.156} = 0.112.$$

Examples. Table 1 gives some examples showing the accuracy with which known quantities of phosphorus can be determined.

Table 1.

Analyses of a solution of primary potassium phosphate.  
20 cc solution contained 1 mg phosphorus.

No. of Exp.	Used of the solution cc	Content of phos- phorus in the solution mg	Amount of n/10 sodium hydroxide used in titration (a) cc	Amount of phosphorus found (a $\times$ 0.112) mg
Without digestion				
1	20	1.000	8.98	1.006
2	10	0.500	4.45	0.498
3	5	0.250	2.24	0.251
4	2	0.100	0.86	0.096
5	1	0.050	0.43	0.048
With acid digestion				
6	20	1.000	8.89	0.996
7	10	0.500	4.38	0.491
8	2	0.100	0.92	0.103
9	1	0.050	0.44	0.049

The accuracy of the method may be seen by comparing the third and fifth vertical columns of the table. It should be noted that in Experiments Nos. 6—9, 5 cc of a 5 % glucose solution completely phosphate-free was added prior to the acid digestion, which was carried out as usual.

Table 2 gives some examples of the agreement to be expected in analyses of proteins containing phosphorus.

Table 2.

Analyses of Protein Solutions.

Exp. No.	Substance used	Quantity of n/10 sodium hydroxide (a) used in titration cc	Amount of phosphorus found $(a \times 0.112)$ mg
1.	Casein	19.10	2.139
2	—	19.05	2.134
3	—	19.05	2.134
4	—	19.13	2.143
5	—	19.11	2.140
6	—	7.00	0.784
7	—	6.99	0.783
8	—	6.94	0.777
9	—	6.99	0.783
10	—	6.93	0.776
11	Globulin of horse serum	1.22	0.136
12		1.18	0.132
13		1.15	0.129
14		1.15	0.129
15		1.19	0.133

For Experiments Nos. 1—5 and 6—10, we used casein solutions, 10 cc being taken for each experiment, and dried as above described.

For Experiments Nos. 11—15, a serum globulin solution with high ammonium sulphate content was used. These experiments may therefore serve as examples of the application of the method in cases where a content of phosphates, or of large quantities of salt, necessitate the precipitation of the protein by coagulation or otherwise before the digestion takes place. After adding 10 cc n/1 sodium acetate + 10 cc n/1 acetic acid, + 10 cc saturated

potassium sodium sulphate solution, and water to 100 cc, the samples were coagulated in a boiling water bath for 15 minutes. The precipitate was filtered off and washed three times on the filter with boiling water, and then carried over by means of water into the Kjeldahl flask, in which, after drying, it was digested<sup>1)</sup> by acid in the usual way.

It will be seen from the last vertical column of the table that the agreement between the different experiments must be considered satisfactory.

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<sup>1)</sup> Even the purest filters contain a little phosphate; the precipitate must therefore be separated off from the filter.

*March 1925.*

# COMPTES-RENDUS

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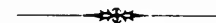
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# STUDIES ON PROTEINS.

BY

S. P. L. SØRENSEN.

## VIII. ON THE SOLUBILITY OF THE SERUM GLOBULINS.<sup>1)</sup>

**D**uring the past 10-12 years, we have carried out, at the Carlsberg Laboratory in Copenhagen, a series of research works to elucidate the chemical and physico-chemical conditions of certain protein solutions. In our first experiments, we used chiefly egg albumin solutions, and we succeeded, as I had the honour to explain at the Naturforskermode in Christiania in 1916, in showing that the views known from the study of real solutions can in many important respects also be applied to solutions of crystallised egg albumin.

Since then, we have extended our investigations to other proteins, and have made comprehensive researches — not yet completed — with the proteins of horse serum. As regards the serum albumin, we found, at any rate in part, similar conditions to those of egg albumin; in the case of the serum globulins on the other hand, we encountered, and are still encountering, great difficulties. These lie firstly in the fact that we have not hitherto been able to get any fraction of the serum globulin in a pronounced crystalline form; further, and not least, in the fact that the globulins, both in the serum itself and in separate globulin fractions obtained from it, do not occur as mixtures of two or more globulins, but as compounds of the same.

As to the nature of the combinations between the globulins, whether we have before us true compounds, as I am most inclined to think, or whether it is a question of solid solutions or

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<sup>1)</sup> The present paper is mainly a reproduction of a lecture delivered on the 11. July 1923 at the 17th Skandinaviske Naturforskermode in Gothenburg.

of a reciprocal adsorption between the different globulins, I shall venture no statement. My object today is to put before you a series of experiments of various sorts, which render it highly probable that such complex globulin compounds do exist, and thus afford an explanation of the remarkable solubility conditions we find in the globulins.

It is generally stated that serum contains two globulins, viz. the Euglobulin — first obtained by Panum — which is insoluble or hardly soluble, and may be precipitated from the serum by sufficient dilution with water, or by adding half its volume of saturated ammonium sulphate solution; and the Pseudoglobulin, soluble in water, which is precipitated by adding to the serum an equal quantity of saturated ammonium sulphate solution. After the globulins have been filtered off, the serum albumin can be obtained in crystalline form from the filtrate by slightly acidulating it.

On the whole, this precipitation system is correct, but great difficulties arise when putting it into practice. I cannot here go into all the numerous proposals which have been made as to alterations in the process of separating albumin and globulin, but will merely state very briefly the method which we have found it best to employ in order to obtain both the globulins and the albumins from a given sample of serum.

As long as it is only a question of getting out the globulins alone, it is best to dilute the serum very considerably at once, prior to the first precipitation with ammonium sulphate, in order to get rid of practically all the albumin and serum colouring matter at the first precipitation. With a view to obtaining the albumin also, however, we precipitated the undiluted serum sample with an equal quantity of saturated ammonium sulphate solution. After filtering off the precipitate, and washing it with a semi-saturated ammonium sulphate solution (noted as (50)), the main quantity of the crystalline albumin can be obtained from the filtrate and the washing liquid by proper acidulation with weak sulphuric acid.

I must here observe that what we understand by the term (50) is a mixture of 50 cc saturated ammonium sulphate solution and (100 ÷ 50) cc water. That is, we do not take in consideration any possible alteration of the volume on mixing, and similarly, in the usual preparatory operations, we assume that the protein has no volume at all. If for instance we dissolve a precipitate washed with (50) in

100 cc water, thus giving a total volume of 250 cc, we reckon that the precipitate retained 150 cc of the washing liquid (50) and that the solution therefore, in the 250 cc, contains in all 75 cc saturated ammonium sulphate solution, and is thus of the composition (30) as

$$\frac{75 \times 100}{250} = 30.$$

The washed globulin precipitate is dissolved in water and precipitated anew to (50), filtered off and washed; this filtrate and this washing liquid will again yield a little crystalline albumin, but the precipitate now contains so little albumin that it is not worth while trying to extract it. The globulin precipitate is therefore dissolved in water, and the solution diluted — if necessary, after previous filtration — to twice the original volume of the serum, when the fractionation of the globulin can be effected by fractionated precipitation with ammonium sulphate. The precipitation was effected by slowly dripping in (70), with good mechanical stirring, and the usual precipitation limits were (30), (35), (40) and (50), though others were also occasionally used. The fractionation was repeated up to 6 times, and carried out in such a manner that the precipitates obtained within like precipitation limits were united and fractionated anew.

I will not weary you with details of these fractionations, but merely note that we succeeded in thus freeing the globulin from all the albumin, but not in completely separating the two globulins one from the other. True, the greater part of the euglobulin is found in the fractions precipitated at the lowest concentration of the ammonium sulphate, but a fraction precipitated between (35) and (40) and repeatedly reprecipitated still contains small quantities of euglobulin, easily demonstrable by dialysis, and all the fractions contain pseudoglobulin as a principal component.

Further separation between the two globulins may be effected by subsequent dialysis of each individual fraction. The dialysis must, to prevent adsorption of water, be carried out under reduced pressure, and to prevent putrefaction and decomposition be effected in ice in an ice box and with the addition of toluol, using also boiled distilled water, and it must be continued until no ammonium sulphate can be found in the dialysate (we generally dialysed for abt. 14 days). On diluting the contents of the dialysis tube with water, filtering off or centrifuging out the precipitate and washing with water, we then obtain the pseudoglobulin in an aqueous solution, the euglobulin remaining undissolved.

Even this operation however, does not give pure products; the aqueous solution of the pseudoglobulin still contains easily demonstrable quantities of euglobulin, while the euglobulin in turn contains considerable amounts of pseudoglobulin. The reason of this lies doubtless in the fact that we have here, as already mentioned, compounds of the two globulins, not mere mechanical mixtures.\* Other writers have also touched upon a similar idea, but without drawing the conclusions from this important fact. I will merely mention that H. C. Haslam<sup>1)</sup> suggests that the difficulty of separating such substances lies perhaps in the fact »that there is some sort of loose chemical combination among them«. The english writer Harriette Chick, who has carried out some of the most thorough and most reliable of recent globulin researches, views the matter in rather a different aspect. Chick realises that euglobulin can be obtained from a pseudoglobulin solution obtained by dialysis and subsequent treatment with water, but she is inclined to suppose that the euglobulin is formed from the pseudoglobulin »by a gradual process of »denaturation«<sup>2)</sup> by which presumably a kind of decomposition of the pseudoglobulin is meant, the phosphorus content of the latter being reduced as the euglobulin is thrown off.

Viewed in the light of our experimental results, the question, then, must probably be answered as follows: The compounds of euglobulin and pseudoglobulin are easily soluble in water and diluted salt solutions as long as these compounds contain plenty of pseudoglobulin, but in proportion as this latter is split off by a simple dissociation process — e. g. by diluting the solution with water — the compounds of the two globulins become less and less easily soluble.

A dissociation process of this nature can be formulated quite schematically as follows:



where E denotes an euglobulin complex or molecule, and P a pseudoglobulin complex or molecule, and where

$$p = b \cdot n + \dots + r$$

and

$$q = a \cdot x + \dots + b \cdot m + \dots + s,$$

<sup>1)</sup> Journ. of Physiol. **32**, 298 (1905) and Biochem. Journ. **7**, 502 (1913).

<sup>2)</sup> Harriette Chick: Biochem. Journ. **8**, 419 (1914).

assuming for the present that all euglobulin complexes, E, and all pseudoglobulin complexes, P, are respectively alike, which, however, is hardly likely to be the case.

On dilution with water, the process moves from left to right, and as  $E_p P_q$  will only be soluble in water when  $q$  is far greater than  $p$ , the less soluble compound  $E_r P_s$  will be thrown off when sufficiently diluted with water.

When small quantities of salt are added, it seems likely that both the more complicated substances  $E_p P_q$  and their dissociation products form compounds with the salt or its ions, and these compounds must be supposed to be present to no inconsiderable degree in non-dissociated form, or, as we may say, as complex, soluble compounds, only slightly giving rise to formation of globulin ions, or at any rate, not forming ions of the hardly soluble globulin compounds. Without some such supposition it would hardly be possible to explain the solubility conditions of these substances.

The more concentrated the salt solution becomes, the greater will be the concentration of these complex undissociated compounds, and at sufficiently high concentrations of salt (differing for the different salts and also for the different globulin complexes) the compound of globulin and salt or one of its components is precipitated.

On the other hand, as the transformations here mentioned are all reciprocal, a dilution of a solution rather poor in salt with water can lead to the formation of one or more of the hardly soluble salt-free globulin complexes, and the precipitation here will be the more abundant the more the solution is diluted.

A very characteristic feature in these transformations is the fact that such precipitation with water or with salt, e. g. with ammonium sulphate, does not take place by reaction solely between the compounds in the solution, but the precipitate already formed takes part in the process when the equilibrium of the solution is disturbed by adding water or salt.

It is a series of experiments to elucidate these conditions which I should like to mention now, only including such details which are necessary to make the process clear.

### A. Is Euglobulin transformed into Pseudoglobulin, or vice versa?

To illustrate this point I have shown in Fig. 1, some experiments with a globulin fraction, reprecipitated four times, each time only including the amount precipitated before (30). This fraction, which contained more than 13 g. globulin nitrogen, should then, according to the view generally adopted, contain chiefly euglobulin. It was found, however, that the fraction after dissolving in water and thorough dialysis, yielded a residue of which 75.87 % dissolved in water by first treatment with water, while further 6.89 % could be extracted by repeated washing with water, leaving only 17.24 % of the original quantity of nitrogen in the insoluble or very slightly soluble precipitate.

I would merely point out that the figures here shown are not those calculated directly from the analyses, but recalculated values, in arriving at which, the quantities removed for analyses, samples and experiments of different character are included, all the figures being, for the sake of convenience, noted as % of the original amount of nitrogen.

The next question then is, whether the precipitate obtained consists of pure euglobulin, and whether the solutions contain only pseudoglobulin.

We will first consider the precipitate (17.24 %) forming the euglobulin fraction. On further washing with water, no essential quantity of pseudoglobulin can be extracted, but if the precipitate be dissolved in 0.6 n potassium chloride solution, and the solution diluted to twenty times its volume (0.03 n KCl) with water, repeating this operation once or twice, we find that altogether more than a third, namely 6.19 %, remains in the solution. The fact is not that the euglobulin is not altogether insoluble in 0.03 n potassium chloride solution, for if the solutions obtained be precipitated with ammonium sulphate, and the precipitate thus obtained — after filtering and dissolving in water — subjected to complete dialysis, then the essential part of the dialytic residue, viz. 5.25 %, will be soluble in water, and only the smaller part, 0.94 % insoluble. Nor is it a case of some pseudoglobulin being carried with or enveloped by euglobulin; the washing with water was too carefully done for this to take place. There can hardly be any other explanation than that we have here a splitting off, a scaling off, of pseudoglobulin particles which had been associated with, combined with, the euglobulin

**Figure 1.** Fraction  $\alpha$ , precipitated before (30). 13.154 g. Globulin-N = 100 %.

<p>Precipit.: 17.24 %  Reprecipitd.: 0.6 n KCl <math>\longrightarrow</math> 0.03 n KCl</p>		<p>Washing water: 6.89 %  precipitated to (50), reprec. to (40)  precip. dissolved and dialysed</p>		<p>Water solun.: 75.87 %  precip. dissolved and dialysed</p>
<p>Precip.: 11.05 %  Sol.: 6.19 %  Precip. to (50), precip.  dissolv. and dialys.</p>		<p>Water solution with slight trace of precip.</p>		<p>Clear water solution with no trace of precip.</p>
<p>Precip.: 0.94 %  Water Sol.: 5.25 %  Reprecip. together  0.6 n KCl <math>\longrightarrow</math> 0.1 n KCl</p>		<p>Fractionated together. Precipitated to (25)</p>		<p>Mother liquor: 68.97 %  precipitated repeatedly to  (50) and to (40)  Precipitate dissolved in a  slight deficit of water.  Sol. centrifuged from prec.</p>
<p>Precip.: 10.60 %  Sol.: 1.39 %  precip. to (50),  precip. dissolved  and dialysed</p>		<p>Precip.: 13.79 %  precip. dissolved; solution precipitated to (50)  precipitate dissolved and dialysed</p>		<p>Prec.: 2.66 %  Sol.: 60.31 %  Prec. did not  dissolv. clearly  in water but in  salt solution.  Solution  dialysed.</p>
<p>Precip.: 0.16 %  Water Solun.: 1.23 %</p>		<p>Slight precipitate  Water solution  precipitated to (20)</p>		<p>Prec.: 0.32 %  Water sol.: 2.34 %</p>
<p>Precip.: 1.30 %  Solution gave precip. on  dilution with water.  Solun. precip. at (14)</p>		<p>Mother liquor: 12.49 %  precipitated to (35)  Prec.: 12.19 %  Mother liq.:  dissolved and  precipitated to  (22)</p>		<p>Mother liquor:  9.78 %  Mother liquor  dialysed</p>
<p>Precip.: 2.41 %  Solun. gave precip. on  dilution with water.  Solution dialysed</p>		<p>Prec.: 2.38 %  Water sol.: 0.02 %  Prec.: 9.76 %  Water sol.: 9.76 %</p>		<p>Prec.: 0.02 %  Water sol.: 9.76 %</p>

particles, and which are, on simple treatment with water, only dissociated off in small quantity, but owing to the disturbance of equilibrium due to addition of salt, are thrown off in greater quantities on treatment with the potassium chloride solution, and are then, on subsequent dilution with water, only partly reprecipitated together with the euglobulin.

This treatment with potassium chloride and precipitation with water therefore also renders the precipitate obtained less and less soluble, and at last, only quite small amounts remain in the solution, when the dilution with water is carried so far that the concentration of potassium chloride is at last only 0.03 n. If, however, the reprecipitation be continued, but the dilution carried only to 0.1 n potassium chloride, then distinct quantities of pseudoglobulin will again be split off. It will be seen from the figure that the amount thus formed is more than a tenth of the quantity, viz. 1.39 % and of this, we had, after precipitation and dialysis, only 0.16 % insoluble in water, the remainder, 1.23 %, being soluble in water.

As experiments with other kinds of precipitate have shown us, there will be nothing to prevent the further continuation of this liberation of pseudoglobulin.

Turning now to the other side of Fig. 1, where the easily soluble fractions are shown, we find that both of them — the aqueous solution as well as the washing water — are precipitated with ammonium sulphate to (50) and then reprecipitated to (40) but not subjected to any fractionation. The precipitates are dissolved in water, and the solutions dialysed, but the residues thus obtained dissolve perfectly clearly in water (washing water fraction did, it is true, show a slight cloudiness). There has thus been no transformation of pseudoglobulin to euglobulin, and as no fractionation was made, there was no disturbance of the equilibrium, so that we cannot expect to find any precipitation of euglobulin. It is quite a different matter when the solutions mixed are fractionated with ammonium sulphate.

On fractionating to (25) one sixth of the total globulin present was precipitated out, and, as will be seen from the figure, euglobulin was easily obtained from this precipitate. On dissolving the precipitate in water, with subsequent dialysis, the residue, when dissolved in water, already yielded a precipitate when precipitated to (20). The precipitate thus obtained dissolved



clearly in water, but the solution gave a precipitate when diluted with water, and was precipitated already at (14). The mother liquor could also, however, as will be seen from the figure, yield small quantities of euglobulin on suitable fractionation and dialysis.

Finally I would call attention to the section of the figure farthest to the right, showing the most easily soluble portion of the whole fraction  $\alpha$ , viz. "mother liquor: 68.97 %". From this most easily soluble portion also it was found possible to gain by no means inconsiderable quantities of euglobulin, the precipitate obtained with ammonium sulphate being dissolved in a small deficit of water. After leaving the mass from one day to the next, and centrifuging the undissolved precipitate from the thick mother liquor, a precipitate was obtained which amounted to only  $1/26$  of the whole amount, but the precipitate was rich in euglobulin, as it did not dissolve clearly in water, but only in salt solutions, and the solution, on dialysis, gave a residue of which no less than  $1/8$  was insoluble in water.

We can, then, we believe, from these and similar experiments, conclude that there is no question of any transformation from pseudoglobulin to euglobulin or vice versa, but that on the other hand, the hardly soluble fraction called euglobulin can, by suitable treatment, be made to yield a certain quantity of pseudoglobulin, while similarly, some euglobulin may be obtained by suitable fractionation of the pseudoglobulin solution.

## **B. Solubility of the Euglobulin in neutral salt solutions.**

The most extensive investigations as to solubility of euglobulin in salt solutions have been made of J. Mellanby<sup>1)</sup> and published simultaneously with the classical works of W. B. Hardy<sup>2)</sup> on globulins.

Mellanby finds that the solubility of euglobulin in neutral salt solutions rises with the concentration of salt as long as the salt solutions are fairly diluted, but he also finds the remarkable fact, likewise noted by Hardy, that the quantity of globulin dissolved at a given salt concentration is,

<sup>1)</sup> Journ. of Physiol. **33**, 338, (1905).

<sup>2)</sup> Journ. of Physiol. **33**, 251, (1905).

approximately, directly proportional to the total amount of globulin used for the solubility experiment.

These experimental results are, on the whole, confirmed by those of our experiments.

In Table 1, and graphically in Fig. 2, will be found the result of two such series of experiments. For the first series we used a sample of euglobulin marked B<sub>2</sub>, obtained from a fraction (35)–(40) by dialysis, treatment of the dialytic residue with water, and washing the precipitate remaining with eight times its volume of water.

**Table 1.**

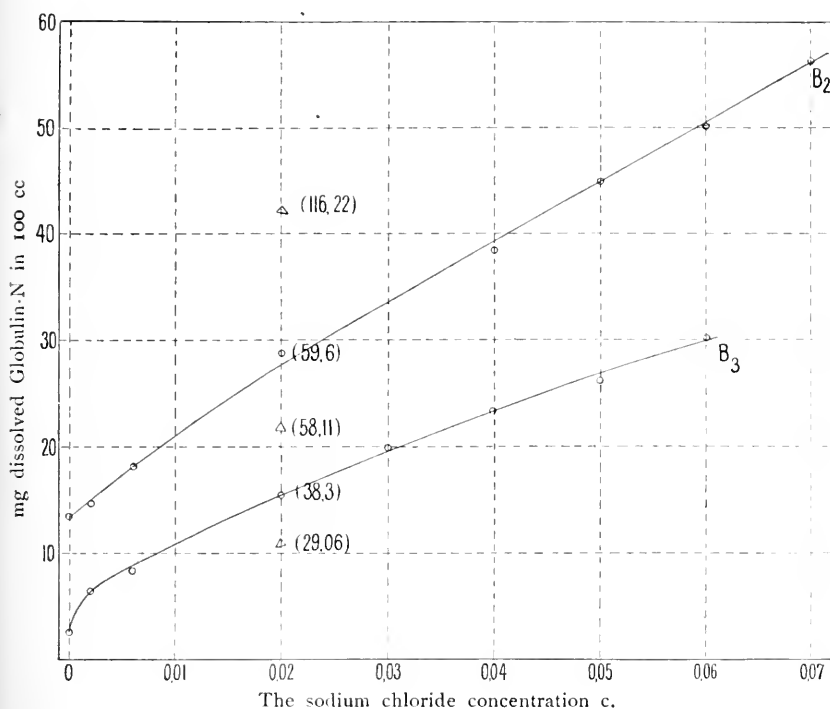
Solubility in sodium chloride solution.

(Precipitation-equilibrium — 48 hours standing — 18°).

Nature of the globulin	Total of globulin-N per 100 cc used for experiment	Concentration of sodium chloride	Quantity of Globulin-N dissolved per 100 cc solution
	mg	c	mg
Obtained from a fraction (35)–(40) by dialysis. The precipitate formed was filtered off and washed once with water, then stirred up with eight times the volume of water and filtered.	59.6	—	13.45
	do.	0.002	14.60
	do.	0.006	18.15
	do.	0.020	28.70
	do.	0.040	38.40
	do.	0.050	44.80
	do.	0.060	50.10
	do.	0.070	56.25
Obtained from B <sub>2</sub> (see above) by stirring with ten times the volume of water and filtering after 24 hours' standing.	38.3	—	2.50
	do.	0.002	6.40
	do.	0.006	8.30
	do.	0.020	15.50
	do.	0.030	19.90
	do.	0.040	23.35
	do.	0.050	26.10
	do.	0.060	30.15
Precipitate B <sub>3</sub> (see above).	29.06	0.020	10.95
	58.11	do.	21.75
	116.22	do.	42.15

For the second series of experiments, we used the same sample of euglobulin, after it had been left to stand for twenty-four hours with ten times its volume of water, and the solution obtained had been filtered off; the precipitate remaining was suspended in water and marked B<sub>3</sub>.

Figure 2.



In the figure here given, the salt concentration (in this case sodium chloride)  $c$  is used as abscissa and the quantity of globulin nitrogen in mg per 100 cc solution as ordinate. From this, and the table together, it will be seen that the solubility of the globulin increases with the concentration of salt, and that sample B<sub>2</sub> is more easily soluble than sample B<sub>3</sub>.

It should here be noted however, that the quantity of globulin suspension B<sub>2</sub> used for each experiment contained 59.6 mg Globulin-N, whereas the corresponding quantity for the B<sub>3</sub> sample was only 38.3. For this reason alone then, in view of Mellanby's results, we should expect to find less globulin dissolved in experiments with B<sub>3</sub> than in those with B<sub>2</sub>. We therefore made

the three experiments noted at the bottom of Table 1, all at the same salt concentration, but with different quantities of the globulin sample B<sub>3</sub>. The results of these three experiments are shown in the figure as small triangles. It is plain enough that the solubility of B<sub>3</sub> depends on the quantity of globulin sample used for the experiment, but it is also evident that the treatment of B<sub>2</sub> with water considerably reduced the solubility.

A really effective purification cannot, however, be obtained by washing with water, but only by dissolving in salt solution and subsequent precipitating with water. As an instance of this we may mention that the remainder of B<sub>3</sub> (about 290 mg Globulin-N in all) was purified by solution in 10 cc 0.4 n sodium chloride solution and diluting the solution to 250 cc, so that the sodium chloride concentration became 0.016 n. After shaking and standing till next day at ordinary temperature, the mixture was filtered, the filtrate analysed and the precipitate again dissolved in 10 cc 0.4 n salt solution, when the precipitation was repeated, etc. The analyses, which are given in Table 2, show the progressive purification and accompanying reduction of the solubility in 0.016 n sodium chloride solution, though here also it must be borne in mind that the quantity of globulin still distributed throughout the 250 cc was diminishing.

**Table 2.** Purification of Globulin fraction B<sub>3</sub>.

Nature of Globulin fraction	Quantity of Globulin-N used for reprecipitation per 250 cc solution	Quantity of Globulin-N dissolved per 100 cc solution (0.016 n NaCl)
	mg	mg
The original Globulin fraction B <sub>3</sub> .....	290	36.30
B <sub>3</sub> after first reprecipitation.....	180	6.15
B <sub>3</sub> after second reprecipitation.....	144	2.60
B <sub>3</sub> after third reprecipitation.....	117	0.75

The table clearly shows that the solubility of B<sub>3</sub> is greatly reduced by reprecipitation, and the decrease in solubility is so great that any difference arising from the fact that the quantity of globulin was not the same in all experiments becomes of minor importance.

In further elucidation of the question I will mention another

series of experiments, where the purification of a globulin fraction is followed in every detail. The euglobulin in question was marked B<sub>II</sub>, and was obtained from a fraction (40)–(45) i. e. from the most easily soluble globulin fraction of all — by dialysis, treatment of the dialytic residue with water and repeated washing of the remainder in water. The precipitate then remaining was dissolved in 15 cc n sodium chloride solution, and 100 cc water added to the solution, producing a very slight precipitate, which was filtered off. The filtrate was then precipitated slowly, and with mechanical stirring, with 1 litre of water, and the precipitate thus obtained (B<sub>III</sub>) sank to the bottom on being left to stand, when the mother liquor was drawn off.

B<sub>III</sub> was used as initial material in the experiment, and was reprecipitated three times by dissolving in sodium chloride solution, dissolving and precipitating with water, and both B<sub>III</sub> and the products obtained by reprecipitation, B<sub>IV</sub>, B<sub>V</sub> and B<sub>VI</sub>, were subjected to an analysis whereby the solubility in 0.02 n sodium chloride solution was determined, three different known quantities of globulin from each fraction being used for the solubility determinations. And finally, the ratio between phosphorus and nitrogen content of the different fractions was determined.

The analytical results are shown in Table 3 and graphically stated in figure 3, where the total quantity of globulin nitrogen used for the solubility experiment serves as abscissa, the amount of globulin nitrogen dissolved per 100 cc solution as ordinate. Both table and figure show that B<sub>III</sub>, though derived from one of the most soluble of all globulin fractions, is, owing to the previous purification, already rather hardly soluble, at any rate, less easily soluble than B<sub>3</sub> (cf Table 2). It will further be seen that the purification of the globulin, details of which are fully set forth in Table 3, not only renders the euglobulin less and less easily soluble, but also renders the solubility approximately constant, and independent of the amount of globulin used in the solubility experiment. This is expressed graphically in the fact that the curves on figure 3 approach more and more nearly to a course parallel to the abscissa axis.

If it were really a question of a single substance, this substance should have a constant solubility in water or in salt solution of a given concentration, ir-

respective of whether the surplus of globulin left undissolved is large or small. As just mentioned, however, euglobulin does not behave in this manner, either after thorough washing with water or after several reprecipitations.

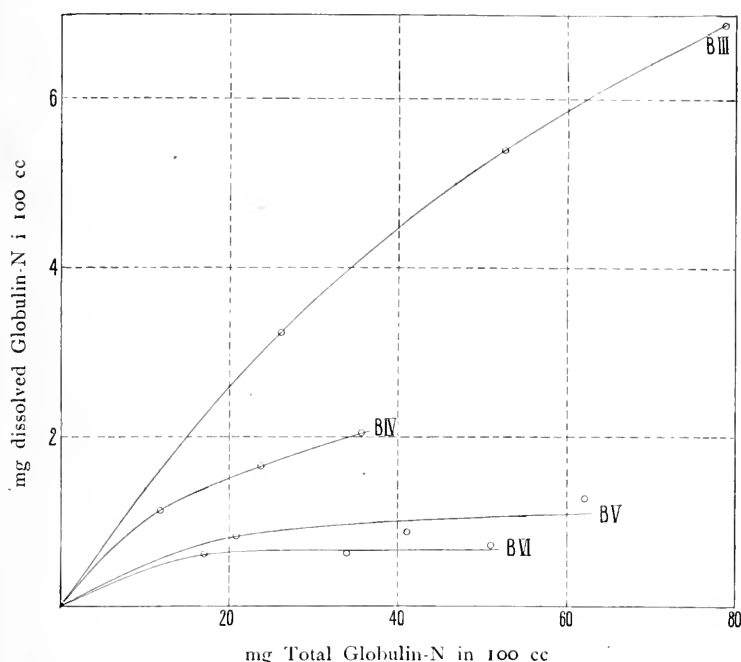
**Table 3.** Purification of Globulin fraction BIII.

Nature of Globulin fraction	Solubility of Globulin fraction in 0.02 n NaCl solution		Phosphorus per g Globulin-N in
	Total Globulin-N used for solubility experiment per 100 cc	Quantity of Globulin-N dissolved per 100 cc solution	
	mg	mg	mg
BIII	26.22	3.23	} 4
(see text).	52.44	5.39	
	78.66	6.89	
BIV	11.92	1.13	} 4
(BIII dissolved in 125 cc water + 15 cc n NaCl precipitated with 1 l water; mother liquor drawn off, washing with 250 cc water, washing water drawn off, BIV suspended in 200 cc water).	23.84	1.64	
	35.76	2.05	
BV	20.71	0.82	} 6
(BIV dissolved in 140 cc water + 30 cc n NaCl, precip. with 1 l water; mother liquor drawn off, washing with 200 cc water, washing water drawn off and BV suspended in 95 cc of water).	41.42	0.87	
	62.13	1.28	
BVI	17.08	0.62	} 10
(BV dissolved in 45 cc water + 35 cc n NaCl, precipitated with 850 cc water, mother liquor drawn off, washing with 190 cc water, washing water drawn off and BVI suspended in 100 cc water).	34.16	0.62	
	51.24	0.72	

Euglobulin can be treated so long and so often with water that the pseudoglobulin complexes which can be dissociated in pure water are really split off, and the remaining substance will consequently show — at least approximately — a constant solubility in water, independent of the amount of globulin employed.

Thus Edwin J. Cohn<sup>1)</sup>, in a series of carefully conducted and recently published experiments, has found that the solubility in pure water of well-washed euglobulin corresponds to about 1.2 mg. of globulin nitrogen per 100 cc. of saturated solution.

**Figure 3.**



We have been able to obtain exactly similar results, but if the purification of the globulin was continued, or was conducted not only by washing with water, but also by reprecipitation by means of neutral salt solutions, the reprecipitated globulin exhibited considerably decreased solubility in water, furthermore, as shown in Table 3, such an adequately reprecipitated fraction Bvi exhibits an approximately constant solubility in 0.02 *N* sodium chloride solution, which is lower than the solubility in pure water stated by Cohn.

However, even a substance such as Bvi cannot be regarded as a pure euglobulin; it exhibits indeed a fairly constant solubility in water and such weak sodium chloride solutions as

<sup>1)</sup> Journ. of Gen. Physiol. 4, 697 (1922).

0.02  $N$ , but in stronger salt solutions, where the dissociation of pseudoglobulin complexes can be carried still further, such a globulin preparation as Bv<sub>1</sub> shows a solubility dependent on the amount of globulin employed. On account of lack of material, we have unfortunately not been able to conduct such a solubility experiment with Bv<sub>1</sub>, but we have done so with another preparation called aa<sub>4</sub>.

The globulin fraction aa<sub>4</sub> was derived from the fraction (precipitated before (30)) mentioned in Fig. 1; aa<sub>4</sub> formed a part of the least soluble precipitate (marked precipit. 10.60 %) but was reprecipitated again by potassium chloride solution. aa<sub>4</sub> contained, per 1 g nitrogen, no less than 46 mg phosphorus.

With this repeatedly precipitated, and therefore very slightly soluble fraction, we made a series of solubility experiments in fairly strong potassium chloride solutions, and with three different amounts of globulin for each concentration of the potassium chloride. The experimental results are given in Table 4, and graphically in figure 4, where potassium chloride concentration is taken as abscissa, and the amount of globulin nitrogen in mg per 100 cc solution as ordinate.

It will be seen both from the table and from the figure that the solubility of aa<sub>4</sub> in these comparatively strong potassium chloride solutions is to a high degree dependent on the amount of globulin used for the experiment, so that we have here precisely similar conditions to those we found in the solubility determinations of less thoroughly purified euglobulin samples in water or dilute salt solutions.

It will further be seen that in these experiments we have reached such concentrations of salt that the solubility of the globulin again begins to decrease, doubtless owing to the incipient precipitation of compounds between the globulin and the salt or one of its components. The middle portions of the curves are shown by dotted lines, as here, at optimal salt concentrations for solubility of globulin, we probably did not have completely saturated solutions; at any rate, in these experiments, the amount of residual precipitate was extremely slight, and we must always reckon with the presence of small quantities of denaturated globulin.

In these experiments, we have a pronounced example of the fact that euglobulin, even when very



Table 4.

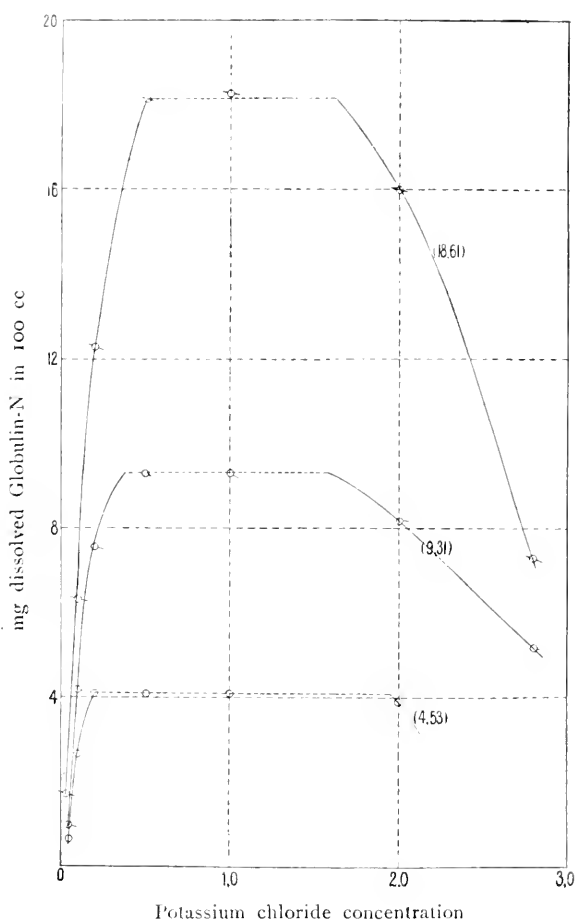
Solubility of Globulin fraction  $\alpha\alpha_1$  in potassium chloride solution. (Precipitation equilibrium — 24 hours' standing — 18°)

Concentration of potassium chloride	When the total of globulin-nitrogen used for the solubility experiment per 100 cc was		
	4.53 mg	9.31 mg	18.61 mg
c	the amount of globulin-nitrogen dissolved per 100 cc solution was		
	mg	mg	mg
0.05 n	0.62	0.98	1.70
0.10 -	2.63	4.22	6.33
0.20 -	4.12	7.57	12.25
0.50 -	4.17	9.27	18.07
1.00 -	4.12	9.27	18.27
2.00 -	3.91	8.18	16.00
2.80 -	—	5.20	7.30

thoroughly freed from pseudoglobulin, can be further decomposed by dissolving in strong potassium chloride solution, when the subsequent dilution with water is so slight that the precipitation of the globulin is only partial. If, on the other hand, abundant water be added, all the euglobulin will be precipitated anew, as the precipitate first obtained will, on the further addition of water, take up and combine with the globulin complexes present in the solution, until a state of equilibrium between the precipitate and the globulin in the solution, answering to the slighter concentration of salt, is reached. That it is really a case of such reciprocal action between the precipitate first obtained and the globulin in the solution, not of continuous precipitation of a uniform precipitate, can be shown by a fractionated precipitation with water. This must be effected in such a manner that the precipitate first obtained, which should contain relatively least pseudoglobulin, and therefore be least soluble, is separated off before any further addition of water takes place.

An experiment to illustrate this point was made with the residue of  $\alpha\alpha_4$ , which was fractionated by dissolving in 0.5 n potassium chloride solution, filtering off a little undissolved, and

Figure 4.



precipitating the filtrate by slowly adding water until the concentration of the potassium chloride was brought down to 0.2 n. After standing in an ice box, the precipitate ( $\alpha_4$  I) had settled on the bottom; the mother liquor was then drawn off and precipitated with water to a potassium chloride concentration of 0.1 n. This gave a precipitate ( $\alpha_4$  II) and a mother liquor which, diluted with water to potassium chloride concentration 0.05 n, gave a further precipitate ( $\alpha_4$  III). All the precipitates were washed with 0.05 n potassium chloride solution and water in such proportion that we had at last all the three precipitates suspended in 0.01 n potassium chloride solution.

The solubility of these three globulin fractions in 0.1 n potassium chloride solution compared with the solubility of the original fraction  $\alpha\alpha_4$  may be seen from Table 5 and Fig. 5.

**Table 5.**

Fractionation of globulin fraction  $\alpha\alpha_4$

Nature of globulin fraction	Solubility of globulin fraction in 0.1 n KCl solution		Phosphorus found per g globulin-N in mg
	Total globulin-N used for solubility experi- ment per 100 cc	Quantity of globulin-N dissolved per 100 cc solution	
	mg	mg	
Original globulin- fraction $\alpha\alpha_4$	4.53	2.63	} 46
	9.31	4.22	
Fraction $\alpha\alpha_4$ I (prec. between 0.5 & 0.2 n KCl)	4.04	0.77	} 57
	8.08	1.08	
Fraction $\alpha\alpha_4$ II (prec. between 0.2 & 0.1 n KCl)	3.75	1.29	} —
	7.49	1.96	
Fraction $\alpha\alpha_4$ III (prec. between 0.1 & 0.05 n KCl)	3.38	2.37	} 32
	6.75	3.96	

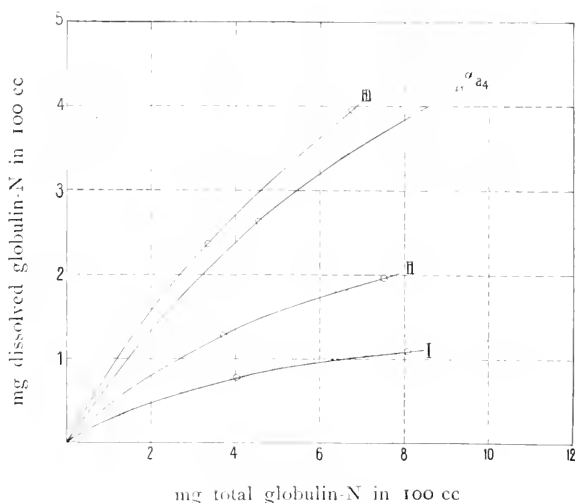
It will be seen both from the table and the figure, where the total quantity of globulin nitrogen used for the solubility experiment is used as abscissa and the quantity of globulin nitrogen in mg per 100 cc solution as ordinate, that the three fractions exhibited widely different degrees of solubility, and that, as was to be expected, Fraction I in particular, but also to a very marked degree Fraction II, showed less solubility; Fraction III on the other hand greater solubility than the initial material,  $\alpha\alpha_4$ .

### C. Is it possible to produce a pure Pseudoglobulin?

One of the first questions which presents itself in the investigation of pseudoglobulin solutions is very naturally this: whether it is possible, by a suitable combination of fractionation and dialysis, to produce a pseudoglobulin of definite characteristic qualities, first and foremost, constant solubility at constant

concentration of hydrogen ions and ammonium sulphate, and further, with a clearly defined acid- and base-binding power with constant osmotic pressure under given circumstances, &c. The next question then will be, whether such constant characteristic qualities are altered with the precipitation limits employed in fractionation of the pseudoglobulin.

Figure 5.



We will here keep to the first mentioned characteristic feature of the pure protein, the constant solubility under certain given conditions, as we can thus obtain important data as to whether we are dealing with a simple substance or a mixture. A solution of a simple, well defined substance must, on precipitation, e. g. with ammonium sulphate at a certain concentration, always give the same protein concentration in the mother liquor, irrespective of the protein concentration of the solution prior to precipitation. If we precipitate a series of samples containing different quantities of the protein solution in question, taking care to keep the concentration of hydrogen ions and of ammonium sulphate at the end of the precipitation uniform for all samples, then the protein concentration should be alike in all the filtrates. Therefore, if we choose a concentration of ammonium sulphate capable of precipitating an essential part of the protein, it will be easy then to observe the presence of any small quantities of a more soluble protein, as the whole of this,

or at any rate the essential part of it, will be present in the mother liquor. This again means, that the protein concentration in the mother liquor will, owing to this impurity, be the greater, the more we have used of the original solution for the experiment.

A necessary condition for the planning and carrying out of such a purity test however is, that the water content of the precipitate be known; otherwise, it will be impossible to calculate how much ammonium sulphate must be added to each sample in order to give them all the same ammonium sulphate concentration, as the solutions containing most of the protein, and therefore yielding most precipitate, will be deprived of more water in precipitation than the rest. In previous investigations we have found that 1 g egg albumin crystallised out with abt. 0.22 g water, the factor whereby the weight of egg albumin nitrogen must be multiplied to give the weight of water-free egg albumin being abt. 6.4, while the factor 7.86 has to be used in order to get the weight of hydrated egg albumin crystallised out. I cannot here go into the question of how these factors are determined, but will merely mention that serum albumin and serum globulin crystallise and precipitate respectively with even more water than egg albumin, the factor for crystallised serum albumin being abt. 8.35 at ordinary temperature and for precipitated pseudoglobulin abt. 8.9 (we generally reckon with 8.88).

We have subjected two different pseudoglobulin samples to a purity test of this sort, both samples having been carefully purified beforehand. The two samples represent pseudoglobulins of widely different solubility, the one, marked Fv, representing the fraction (33.3)-(45), and the other, marked  $\alpha B_4b$ , precipitated between (22)-(35).

Fv is derived from a globulin fraction B, precipitated four times between (35) and (40), then freed from less soluble globulin by dialysis, and the easily soluble portion reprecipitated four times between (33.3) and (45).

$\alpha B_4b$  is derived from the fraction  $\alpha$ , precipitated before (30) mentioned in Fig. 1; the less soluble portion was removed by dialysis and subsequent treatment with water, and the soluble part so fractionated as to separate out the least soluble part,  $\alpha B_4b$  forming the fraction precipitated between (30) and (35) and reprecipitated four times, afterwards precipitated twice in a fairly strong solution between (22) and (35).

Details of the purity tests are given in table 6, where the data requisite for understanding of the process are also noted.

**Table 6.**

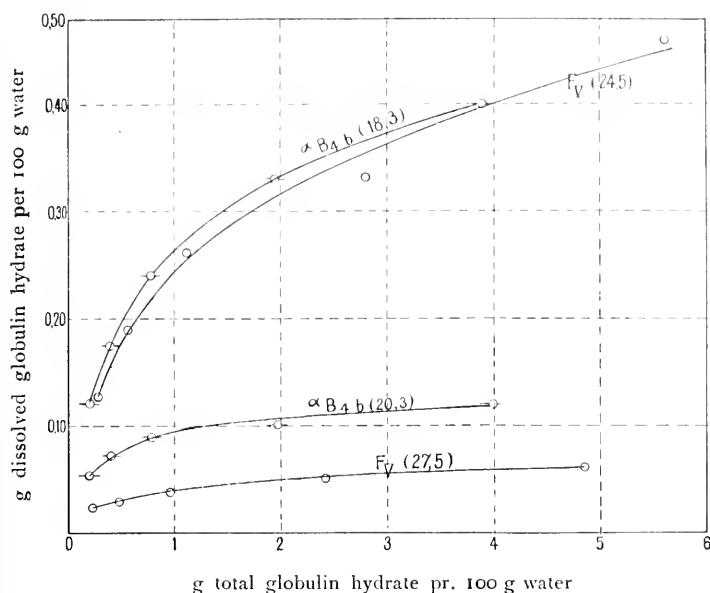
Precipitation at same concentration of ammonium sulphate  
but different concentrations of globulin.

Globulin mark and No. of experiment	Experimental mixture contained per 100 g water globulin hydrate (factor: 8.88) in g	Filtrate contained per 100 g water	
		Ammonium- sulphate in g	Globulin hydrate (factor: 8.88) in g
Fv 1.....	0.280	24.555	0.127
- 2.....	0.559	24.470	0.189
- 3.....	1.119	24.585	0.261
- 4.....	2.797	24.516	0.333
- 5.....	5.596	24.541	0.460
- 6.....	0.242	27.513	0.023
- 7.....	0.483	27.517	0.028
- 8.....	0.966	27.531	0.038
- 9.....	2.416	27.534	0.050
- 10.....	4.833	27.592	0.061
$\alpha B_4b$ 1.....	0.195	18.297	0.121
- 2.....	0.391	18.301	0.175
- 3.....	0.781	18.318	0.239
- 4.....	1.953	18.312	0.330
- 5.....	3.906	18.310	0.400
- 6.....	0.197	20.291	0.053
- 7.....	0.395	20.320	0.072
- 8.....	0.788	20.295	0.088
- 9.....	1.972	20.305	0.100
- 10.....	3.944	20.316	0.119

Both with Fv and with  $\alpha B_4b$  we made ten precipitation experiments with two concentrations of ammonium sulphate and five different concentrations of protein, varying in about the proportion 1:2:4:10:20. In the case of Fv, the total volume after precipitation was abt. 175 cc, that of  $\alpha B_4b$  abt. 200 cc. The quantity of ammonium sulphate per 100 g water was, in the precipitation of Fv abt. 24.5 g and abt. 27.5 g respectively, the corresponding values for  $\alpha B_4b$  being abt. 18.3 g and abt. 20.3 g. It will be seen from the last vertical column but one in the table, (quantity of ammonium sulphate) that there is good agreement between the separate series of ex-

periments, which shows that the factor 8.88 is serviceable enough in calculating the composition of the globulin precipitate. The concentration of hydrogen ions in the filtrate answered to  $p_{H^+} = 4.9-5.0$  for Fv and  $p_{H^+} = 4.8-4.9$  for  $\alpha B_4b$ . The experimental results are shown graphically in Fig. 6, where the total quantity of globulin hydrate in g. per 100 g. water is used as abscissa, and the quantity of globulin hydrate in the filtrates, in g. per 100 g water as ordinate.

Figure 6.



It will be seen both from the table and the figure that there is no question of pure substances, showing a constant solubility independent of the original protein concentration; if this were the case, the graphical reproduction of the experimental results would have appeared in the form of straight lines, parallel with the abscissa axis.

Nor is it a question of substances containing small quantities of more easily soluble proteins, for in such case, the graph would have given straight lines at an angle with the abscissa axis, the angle being the greater as the quantity of easily soluble impurity increased.

We have not — and it will perhaps be difficult to procure —

sufficient analytical data for mathematical treatment of the problem, but from a purely qualitative point of view, the experimental results are easily explained if we suppose the pseudoglobulin precipitate also to be of the above noted composition  $E_p P_q$ , where  $q$  is far greater than  $p$ , and the globulin complex therefore is soluble in water as well as in dilute salt solutions.

On precipitating such a solution with ammonium sulphate, we get first of all complexes rich in euglobulin, and not until ampler quantities of the salt are added, will complexes poor in euglobulin, and also the pseudoglobulin containing no euglobulin, be precipitated, as during precipitation, a reciprocal action takes place between the globulin first precipitated and that still remaining in the solution. But the result of this will be that the quantity of pseudoglobulin remaining in the solution on precipitation will increase, though not proportionally to the total amount of globulin, as the quantity of precipitate also increases, and thus also the amount of pseudoglobulin which it binds; graphically then, we obtain, not straight lines, but curves bending off towards the abscissa axis.

In the order to demonstrate further that in such precipitation of pseudoglobulin with ammonium sulphate, a fractionation takes place, which on continued precipitation appears as reciprocal action between the first-formed precipitate rich in euglobulin and the globulin still remaining in the solution, we have made some experiments in which we first precipitated a certain amount and then further precipitated part of the solution with precipitate suspended, another part being filtered and the filtrate further precipitated. If the concentration of hydrogen ions and of ammonium sulphate were otherwise equal, we should expect that the concentration of globulin in the mother liquor would be least in that portion where the precipitation was effected without filtering off the first precipitate, providing the supposition be correct that the presence of this precipitate would occasion a binding of some of the globulin in the solution.

This actually proved to be the case, and I will briefly demonstrate the same by going through one of the series of experiments.

For these experiments, we used a globulin fraction A, precipitated four times between (30) and (40) and then five times between (30) and (36.2).



3200 cc of solution of A, containing abt. 7 g. globulin hydrate per 100 cc, was precipitated slowly and with mechanical stirring to abt. (32), a sample was then taken, of 1000 cc solution with suspended precipitate (marked Aa) and the remainder further precipitated to abt. (34) when a sample of 830 cc solution with suspended precipitate (marked Ab) was taken, and so on.

The samples were left to stand at 18°—19° in covered cylinder glasses with plenty of toluol, and with good and repeated stirring until next day, when they were placed in flasks with close-fitting stoppers. After standing again, with good and repeated shaking, small samples were taken at different times for filtration and analysis of the filtrates. It was found, as was to be expected, that the precipitation continued when the samples were left to stand, though very slowly; a period of 6 days' standing was taken as suitable for all samples.

The quantities not used for analysis were filtered, and the filtrates precipitated further in the same manner as above mentioned in the case of the stock solution, the various samples drawn off during further precipitation of the filtrate from Aa being marked as aa, ab, ac and ad respectively, while those from the further precipitation of the filtrate from Ab were marked respectively ba, bb, bc and so on.

The analytical results are shown in Table 7, and graphically in Figure 7, where the filtrates' content of ammonium sulphate and globulin hydrate respectively per 100 g water are shown as abscissa and ordinate.

The curves in Fig. 7 show very clearly the influence of a precipitate already formed on the further precipitation of a pseudoglobulin solution with ammonium sulphate. The curve farthest to the left (the lowest curve) shows the precipitation results of the solution A, i. e. precipitation where a previous precipitate is already present; it will be seen from the figure that the precipitation under these conditions gives the lowest globulin concentration in the filtrate at the same concentration of ammonium sulphate. The upper curve gives the precipitation results for the samples from filtrate Aa, i. e. that from which only the very first precipitate, the richest in euglobulin, was removed; this precipitate will of course be particularly capable of binding more pseudoglobulin, while the power decreases as the precipitation goes on. Therefore, the curve for

Table 7.

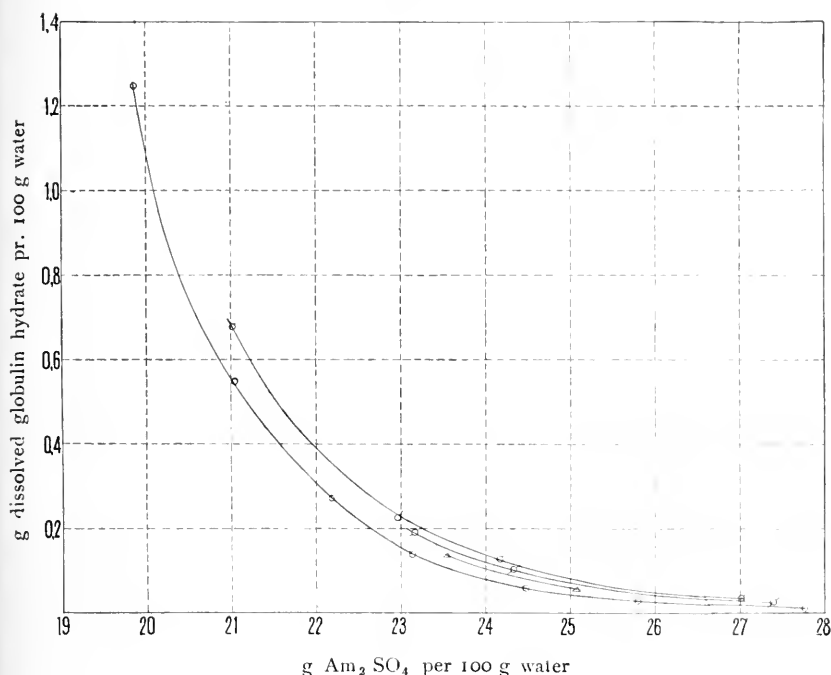
Reciprocal action between the pseudoglobulin in the precipitate and that in the solution on precipitation with ammonium sulphate.

Precipitation mark	Filtrate contained per 100 g water	
	Ammonium sulphate in g	Globulin hydrate (factor: 8.88) in g
Aa .....	19.861	1.254
Ab.....	21.051	0.545
Ac.....	22.178	0.274
Ad.....	23.145	0.138
Ae.....	24.442	0.066
Af.....	25.804	0.029
Ag.....	27.787	0.011
aa .....	21.029	0.678
ab .....	22.956	0.222
ac .....	24.183	0.126
ad .....	27.030	0.032
ba .....	23.157	0.185
bb .....	24.327	0.102
bc .....	27.389	0.026
ca .....	23.521	0.134
cb .....	25.083	0.058
da .....	25.625	0.039
ea .....	27.303	0.013
fa .....	26.915	0.015

the a precipitations (aa, ab ac and ad) must lie uppermost, farthest from the A curve, while the b curve &c. lies nearer this.

Before leaving this subject I would like to emphasize, that I am perfectly aware that some of the phenomena I have noted come under what is generally termed mechanical carrying down or adsorption, while others come under the so-called "protective colloid" category. That I have not included these terms in my remarks is due to the fact that I wished to get somewhat closer to the question than is possible with these very elastic but far from precise classification terms.

Figure 7.



Looking back over the experiments I have put before you to-day, it will be seen that we can draw one common conclusion from them all, to wit, that we have not been dealing with either pure euglobulin (E) or pure pseudoglobulin (P). All our globulin preparations, even the best purified, exhibited qualities which must characterise them as easily dissociable compounds, containing both euglobulin and pseudoglobulin, and therefore all coming under the general formula  $E_p P_q$ ; the solubility of these substances in water and salt solutions was less, the greater  $p$  might be supposed to be in proportion to  $q$ .

Nor do we find in the literature any statements seeming to show that other investigators have had pure euglobulin or pure pseudoglobulin to work with, though we do find statements as to the presumable character of these substances. Attention has been paid more especially to the phosphorus content, and it has been held, that the total phosphorus content of a globulin fraction should be ascribed to the euglobulin, the pseudoglobulin being free of phosphorus. On the whole, this agrees with our

results, as the proportion between phosphorus and nitrogen increases the less soluble a globulin fraction becomes (see for instance fractionation of  $\alpha\alpha_4$ , Table 5) and the purest pseudoglobulin fractions we have had to deal with contained only quite minimal quantities of phosphorus.

Harriette Chick <sup>1)</sup> even believes her experiments warrant the conclusion that euglobulin is probably only "a mechanical complex consisting of a protein (pseudoglobulin) and a lipid" and offers several good arguments in support of this view, inter alia that she succeeded in producing "artificial euglobulin" by mixing an aqueous lecithin emulsion with a salt-free pseudoglobulin solution.

Wolfgang Pauli <sup>2)</sup> who, like Chick, has made a series of fine investigations with the serum proteins, has recently stated as his view, but without further particulars, that serum contains only one protein soluble in water, viz. the albumin, while the pseudoglobulin is only to be regarded as euglobulin kept in solution by quite small quantities of electrolyte.

I cannot here go into the question of what may be urged in support of one or the other of these views, but will merely say, that in my opinion, the case is hardly so simple. I am most inclined to believe that in the formula  $E_p P_q$  we must reckon that we have to deal with different complexes E or P respectively, but a great deal of experimental work will still be required to decide the point.

Finally, I would once more draw attention to what I consider the main result of the experiments here described, namely, the proof furnished by many different ways that reciprocal action takes place between the protein complexes of which the different globulin fractions consist, with the formation of easily dissociable compounds, both when dealing with substances in solution, and with solutions with suspended precipitate mixed.

As with a mixture of amino-acids, whose amphoteric character makes it possible for reciprocal binding to take place, rendering it most difficult to separate such a mixture, so also

<sup>1)</sup> Biochem. Journ. 8, 404 (1914).

<sup>2)</sup> Sitzung der math.-naturw. Klasse der Akademie der Wissenschaften in Wien, (26/1 1923).

mixtures of proteins will be able to form compounds one with another, and possibly, in the formation of such large, easily dissociable complexes, we have the explanation of many of the peculiar qualities of the concentrated protein solutions.

I wish to express my best thanks to Mrs. Margrethe Sørensen, and Messrs. Dr. phil. Carsten Olsen and Cand. polyt. K. Linderstrøm-Lang, for valuable assistance in carrying out the experiments here described.

*July 1923.*

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COMPTES-RENDUS  
DES TRAVAUX  
DU  
LABORATOIRE CARLSBERG

16<sup>ME</sup> VOLUME.  
EDITION FRANÇAISE.  
1925—1927



COPENHAGUE  
EN COMMISSION CHEZ H. HAGERUP  
IMPRIMERIE DE THIELE

1927





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# I. ON THE SOLUBILITY OF CASEIN IN HYDROCHLORIC ACID.

BY

K. LINDERSTRÖM-LANG AND SAKUJI KODAMA.

## Introduction.

The following work is a continuation of the investigations carried out at the Carlsberg Laboratory a year ago by Kinsuke Kondo<sup>1</sup>). At the instigation of one of the present writers, Kondo commenced a series of experiments for determination of the solubility of casein in hydrochloric acid, but was unfortunately obliged, owing to lack of time, to discontinue the work before it was completed. The present writers have therefore taken up the experiments anew, and with a somewhat different method from that employed by Kondo.

Kondo's determinations of the solubility of casein in hydrochloric acid revealed the phenomenon that the solubility, at a given concentration of acid, decreased as the quantity of casein present in the form of precipitate increased. The method of these experiments was as follows: Varying quantities of casein (1–4 g) were placed in 100 cc of a hydrochloric acid solution of known concentration, shaken for 24 hours at 18°, and the amount of nitrogen in the solution centrifuged off then determined. It was natural therefore to explain the phenomenon above noted as due to a binding of hydrochloric acid to the precipitate, the more so as such binding of acid to proteins has been observed by several authors notably in the investigations of J. Sjöqvist<sup>2</sup>) and also in those of others since, for casein specially in the papers by L. L. van Slyke and E. B. Harts<sup>3</sup>) and L. L. van Slyke and D. D. van Slyke<sup>4</sup>).

<sup>1</sup>) »Studies on Casein«. *Comptes rendus du Lab. Carlsberg*. **15**, Nr. 8 (1925).

<sup>2</sup>) *Skand. arch. f. Physiol.* **5**, 277, **6**, 255 (1895).

<sup>3</sup>) *Amer. Chem. Journal*, **33**, 461 (1905).

<sup>4</sup>) *Ibid.* **38**, 383 (1907) — *Journ. Biol. Chem.* **4**, 259 (1908).

We have therefore, in our continuation of these experiments, endeavoured to get nearer the root of the problem, by determining the amount of acid in the solution saturated with casein at 18° after centrifuging off the precipitate. At the same time, we measured the activity of hydrogen ions, and in the main experiments, also the activity of chlorine ions, electrometrically.

As regards the actual principle of the method for determination of solubility, there were, as will appear in the following, very essential difficulties in the employment of the method indicated above, as it was found impossible to attain equilibrium by merely shaking the casein and the hydrochloric acid solution together. We therefore adopted another method, first dissolving the casein completely in a surplus of HCl or NaOH, and then, by partial neutralisation of the acid, or by neutralisation of the base with a surplus of acid, obtaining a »fresh« state of equilibrium, with newly formed precipitate capable of reaction. By this means, we succeeded in obtaining a stable, reproducible equilibrium.

The result of these experiments showed a certain relation between the amount of acid bound per unit of nitrogen in the precipitate and the activity of hydrogen ions and that of chlorine ions in the solution. Furthermore, we found a solubility of casein far exceeding the values previously found, while at the same time, this solubility was seen to be peculiarly dependent on the quantity of precipitate formed, a feature which may be explained by the supposition that casein is a mixture of different substances.

The present work has thus connection with the investigations on the qualities of serum globulin carried out during the past few years at this laboratory by S. P. L. Sørensen and assistants<sup>1)</sup>. In these investigations, the solubility determination was used to a great extent as a purity test for proteins, as a means for definition of the same, and, as will be seen from the last part of the present work, it has also been of the greatest importance in these casein investigations. We shall not here discuss the points of similarity between the conditions of solubility in the globulins and casein, as this comparison is to be found in the concluding section of the work; we should like, however, here to express

<sup>1)</sup> Lecture delivered at the 17th Meeting of Scandinavian Naturalists, Gothenburg 1923; Compt. rendus du Lab. Carlsberg. **15**, No. 11 (1925); Journ. Amer. Chem. Soc. **47**, 457, (1925).

our hearty thanks to Prof. Sorensen, for giving us time and opportunity to carry out these extensive investigations, as well as for his valued advice and encouragement in the course of the work.

### 1. The Casein used and Method of Preparation.

The casein used in our experiments was the same as that employed by Kondo. We therefore give his analysis figures (Stud. on Casein, l. c)

Water.....	9.43 %
Ash.....	0.15 % of dried casein
Nitrogen.....	15.46 % - — —

$$F = 6.47.$$

F is the factor by which the amount of nitrogen must be multiplied to give the corresponding weight of dry casein.

This casein was purified by the following method (see Studies on Casein).

100 g casein was mixed with 2000 cc water, to which was added, slowly and with powerful stirring, 3370 cc 0.02 n NaOH, whereby the casein was dissolved, the solution having a concentration of hydrogen ions answering to  $\text{pH} = 7$ . Filtration then followed, and the calculated quantity of hydrochloric acid was added, likewise in the form of 0.02 n HCl, fairly rapidly, and with violent stirring. This certainly precipitated most of the casein in large flakes, but part remained in a colloidal state in the solution, which was very milky and opalescent. A little experiment in which we took samples from the casein mixture and added to the samples varying quantities of 0.01 n HCl, or 0.01 n NaOH, showed that it is necessary to add a slight surplus of acid in order to get the casein completely flocculated out, and render the filtrate clear as water, without any opalescence. The concentration of hydrogen ions in the filtrate was then abt. 4.5 and in the subsequent precipitation, the mixtures of casein in the filtrate were always regulated to this concentration of hydrogen ions. The casein thus precipitated was washed three times by rubbing in a mortar with 3 times 6 litres of 0.01 n NaCl.

The casein will be noted in the following as II or III, these terms referring to the qualities of the different samples according to the arrangement as under:

II. Trade casein, Kahlbaum's, »nach Hammarsten«, reprecipitated twice as above described.

Filtrate from last precipitation with HCl:  $p_{aH} = 4.49$ . 2.44 mg casein-N per 100 cc.

III. Trade casein, as above, reprecipitated three times.

Filtrate from last precipitation with HCl:  $p_{aH} = 4.44$ . 3.98 mg casein-N per 100 cc.

After the final treatment with NaCl, the casein was washed with water, mixed with water and toluol and stored with ice in an ice-chest:

Suspension II. abt. 1.8 litres. 0.74 g casein-N per 100 cc.

Suspension III. abt. 1.6 litres. 0.82 g casein-N per 100 cc.

The Suspension II + III used for the main experiment is a mixture of II and III (600 cc III and 1200 cc II) prepared by mixing the precipitates carefully and rinsing in water till they were free from chlorine.

Suspension II + III. abt. 1.9 litres. 0.63 g casein-N per 100 cc.

## 2. The Analytical and Electrometrical Methods employed.

### *a. Determination of Nitrogen.*

We used the Gunning-Arnold method, modified by A. C. Andersen and B. Norman-Jensen<sup>1</sup>).

### *b. Determination of Chlorine.*

In order to determine the quantity of chlorine in a casein solution, we proceeded as follows: To the casein sample, containing a suitable quantity of chlorine (answering to 5—10 cc 0.1 n silver nitrate solution) was added 5 cc 5 n  $\text{HNO}_3$  and 5 cc 2 n  $\text{H}_2\text{SO}_4$ , with water to 100 cc. This was heated to 40° on a water bath, when the casein precipitated by the acid collected in the form of a slightly yellowish, flaky precipitate, easily filtered off. The precipitate was rinsed with warm, dilute nitric acid, and the filtrate and rinsing water precipitated with a surplus of silver nitrate solution of known quantity and concentration. The solution was now boiled for a couple of minutes, whereby the silver

<sup>1</sup> 113. Beretning fra Statens Forsogslaboratorium (1923).

chloride, which had a tendency to form colloiddally, especially when the quantity of chlorine was small, was liberated in rather fine particles (never in a cheesy mass as in pure solutions) and filtered off. Titration otherwise after Volhard.

The idea of this was to precipitate the casein at the high acid concentration, and, simultaneously with the nitric and sulphuric acid, force out the quantity a hydrochloric acid adsorbed on the precipitate formed. That we succeeded in so doing is evident from the following test experiment:

Table 1.

Experiment.....	0	1	2	3	4
cc 0.1 n HCl.....	10.00	10.38	10.75	11.50	13.00
g casein (unpurified nach Hamm.).....	0	0.025	0.05	0.10	0.20
addet 0.1 n AgNO <sub>3</sub> cc	15	15	15	15	15
Tit. back with 0.1 n KSCN cc.....	4.99	4.70	4.30	3.52	2.08
Found 0.1 n HCl cc..	10.01	10.30	10.70	11.48	12.92

*c. Determination of hydrochloric acid content in a casein solution.*

In a casein solution containing, besides HCl, also sodium chloride, with a concentration high in proportion to that of the hydrochloric acid, a chlorine titration is not accurate enough to determine the amount of HCl. Direct acid titration must here be used instead, choosing an indicator with range at the isoelectric point of casein,  $p_H = 4.8 - 5.2$ . We titrated with 0.01 n NaOH and used a mixture of methyl red and methylene blue as indicator (generally used for acid titration in the Kjeldahl determination). All solutions were diluted 10 times, and the terminal point of the titration was fixed by means of a test with known quantity of hydrochloric acid and a quantity of casein answering more or less to the casein content of the experimental liquors. The colour tint shown by this test experiment after adding the calculated quantity of base and leaving it to stand was used as the standard, and the experimental liquids titrated to the same shade. Our object in diluting to this extent was to reduce the salt concentration of chlorine ions, and thereby reduce the quantity of acid bound by the precipitate at the terminal point of

the titration as far as possible (see p. 40). And a series of experiments with known quantity of hydrochloric acid and different concentrations of casein showed that this quantity of acid bound really was slight when we only titrated to  $p_H = \text{abt. } 5.2$  (answering to the above noted reddish grey shade), so that the casein concentration had no essential effect on the experimental results.

Table 1a.

Experiments.....	0	1	2	3	4	5
cc 0,01 n HCl.....	10	10	10	10	10	10
g casein.....	0	0.025	0.05	0.1	0.2	0.4
diluted to.....	200	200	200	200	200	200
Used for Titr. cc abt.						

0.01 n NaOH .....	9.32	9.35	9.34	9.28	9.15	8.8
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The highest casein content in the samples taken for titration in the experiments noted on pp. 15 and 27—30 was 0,15 g.

#### *d. Determination of hydrogen ion activity.*

For this, we used the Biilmann quinhydrone electrode<sup>1)</sup>. After Lester<sup>2)</sup> in a work on the concentration of hydrogen ions in milk, had tried using this electrode and obtained excellent results, especially with buttermilk, where the magnitude of  $p_H$  was favourable for the quinhydrone measurement (4.7), we made a few experiments with a view to ascertaining whether there might be any demonstrable protein error in the quinhydrone in the acid casein solutions we were working with. (Owing to the slight salt concentration in our solutions, the salt error of the electrode<sup>3)</sup> could not make itself felt). This was found not to be the case, as the two following elements:

- I. Pt. Quinhydrone, 0.01 n HCl,  $H_2$  (1 Atm.) Pt. (18°).
- II. Pt. Quinhydrone, 0.05 n HCl + 0.5 — 1 g Casein in 100 cc,  $H_2$ , Pt. (18°)

both gave as the mean the same value, 0.7038 volts. The quinhydrone used was prepared according to Biilmann and H. Lund<sup>4)</sup> and dried for two days between filter papers. It is

<sup>1)</sup> Ann. de Chimie **15**, (9), 111 1921.

<sup>2)</sup> Journ. Agricult. science **14** 4), 634, (1924).

<sup>3)</sup> Compt. rendus Lab. Carlsberg **14**, N. 14.

<sup>4)</sup> Ann. d. Chimie **15**, (9), 321 1921.



possible that some of the quinone has evaporated during this drying period as it seems from the low value, 0.7038 (0.7045—0.7048 is the normal, cf. Biilmann and <sup>1)</sup>), and in order to correct for any further evaporation during the time our experiments lasted (six months) and for alterations in the calomel electrode, we measured from time to time the potential of the following element:

III. Pt. Quinhydr., 0.01 n HCl, 0.09 n KCl / 3.5 n KCl / cal. electr. at 18°.

Calling this potential — corrected for the diffusion potential —  $E_k$ , and the potential of an element such as III with an arbitrary experimental liquid in place of the HCl-KCl mixture E, then the  $pa_H$  <sup>1)</sup> in the experimental liquor will be determined by

$$pa_H = \frac{E_k - (0.3357 - 0.4556) - E}{0.0577} = \frac{E_k + 0.1199 - E}{0.0577} \quad (1)$$

independently of alterations in the quinhydrone and in the calomel electrode.

This method has enormous advantages. It is much quicker than the usual one, and, a point of still greater importance, it is more accurate. It was very rarely that two parallel determinations differed more than 0.4 millivolt, and the potential remained very constant for several hours.

#### *e. Determination of chlorine ion concentration.*

This has been described, in all essentials, in the work by K. Kondo (l. c) where also the formula

$$pa_{Cl} = \frac{0.0674 - E}{0.0577} \quad (18^\circ) \quad (2)$$

for calculating  $pa_{Cl}$  is noted. E is the potential of the following element:

IV. Hg, HgCl, 0.1 n KCl/3.5 n KCl/Experimental liquid, HgCl, Hg.

### **3. Preliminary Experiments.**

With regard to the solubility of casein in hydrochloric acid, the scattered and uncertain knowledge we have on this point

<sup>1)</sup> Compt rendus Lab. Carlsberg **15**, No 6, p. 38 and 39 (1924).

may well stimulate systematic research. It is not clearly apparent from Kondo's work<sup>1)</sup> whether there was equilibrium or not when the shaking of the casein with hydrochloric acid was discontinued after 24 hours. Loeb's work, again,<sup>2)</sup> leaves it still uncertain whether the quantity of hydrochloric acid which must be present in 100 cc of water in order to dissolve 1 g of casein has not been estimated at too high a value owing to the low velocity of dissolution of casein. T. B. Robertson has carried out some experiments<sup>3)</sup> in which he first dissolves the casein in a surplus of sodium hydroxide, and then adds enough acid to make the casein (1.25 g in 100 cc) first precipitate and then just dissolve, this point being determined by means of a refractometer. By this method, he gets a value for the solubility (vide infra) considerably higher than Loeb's, although there is sodium chloride in the solution, formed during the neutralisation of the base, and sodium chloride has a pronounced depressing effect on the solubility of casein on the acid side of the isoelectric point. It is evident from this that newly precipitated casein has a high power of reaction, dissolving rapidly in hydrochloric acid, whereas the dried casein, as used by Loeb and Kondo, is slow to react, and the period of shaking was not sufficient to establish equilibrium.

Loeb .....	1 g casein dissolves in 100 cc 0.006 n HCl
Kondo .....	1 - — — - — — 0.009 - —
Robertson .....	1 - — — - 80 — 0.004 - —

For further investigation of the point we carried out three experiments with different periods of shaking, as follows. In the first experiment, 40 cc of suspension III was placed in a medicine bottle with 12 cc 0.1 HCl and 148 cc water (weight 195.80 g). The whole was then shaken in a thermostat for 24 hours at  $18^{\circ} \pm 0.1^{\circ}$ , whereby the casein particles swelled out and the mixture assumed the form of a highly viscous semi-transparent solution in which no distinct grains could be observed; when centrifuged at 3000 revolutions per minute it separated into a

<sup>1)</sup> l. c. pag. 1.

<sup>2)</sup> Proteins and the Theory of Colloidal Behavior. New York, 1922, pag. 54, 266—274.

<sup>3)</sup> The Physical Chemistry of Proteins. (Longmans, Green, Co. 1920) pag. 97. Journ. of Phys. Chem. 13, 469 (1909).

thick, sticky precipitate and a small quantity of opaque, slightly viscous solution. Filtration was impossible. We therefore used a larger centrifuge, giving 10000 revolutions per minute, and after centrifuging for a quarter of an hour we succeeded in getting out  $\frac{3}{4}$  of the mixture as a clear, very slightly viscous but somewhat opalescent solution,  $\frac{1}{4}$  being a firm, gluey mixture of precipitate and solution. We ascertained by experiment that no essential advantage was gained by increasing the number of revolutions or period of centrifuging, and we have therefore in all subsequent experiments followed the method here described.

Of the 200 cc of mixture, 100 cc was centrifuged. The room in which the centrifuge stood was kept as nearly as possible at  $18^{\circ}$  (the temperature here is not of such great importance, owing to the slowness of the dissolving processes and the short period of centrifugation). 100.52 g of mixture lost 1.37 g in weight by evaporation during centrifugation. Suitable samples were taken from the solution for determination of chlorine and nitrogen (the samples were weighed) and the results found were corrected for evaporation by multiplying by  $100.52 - 1.37 = 99.15$  and dividing by 100.52. The activity of hydrogen ions was also measured.

The remainder of the mixture was used for direct determination of chlorine and nitrogen in precipitate + solution, whereby, on subtracting the chlorine and nitrogen content of the solution from that of the mixture we can get an idea of the composition of the precipitate. In this however, there is the inevitable error due to the fact that 100 g of mixture does not contain 100 g of solution, but somewhat less, as the precipitate also weighs something (and we must of course first subtract the amounts of chlorine and nitrogen in equal quantities of precipitate and solution one from another). The only correction which can be applied is obtained by multiplying the nitrogen content of the precipitate so far found by the factor 6.47, valid for dry casein (see p. 3) and taking the value thus obtained as the weight of the precipitate. As this correction will not at the outside be over 1%, we have disregarded it in these preliminary experiments, the more so as it cannot be regarded as of any exact value, since the precipitate has bound water, and the true correction would thus be greater.

The second and third experiments were carried out in precisely the same manner as the first one, save that the period of

Table 2.

Period of shaking, in hours . . . . .	24	48	72
cc 0.1 n HCl added per 100 g mixture = mg-equiv. in 1000 g . . .	6	6	6
Chlorine in mixture. mg-equiv. in 1000 g . . . . .	6.85	6.92	6.75
Chlorine in solution. mg-equiv. in 1000 g . . . . .	5.04	5.58	5.90
Nitrogen in mixture. mg-equiv. in 1000 g . . . . .	118.3	118.2	115.8
Nitrogen in solution. mg-equiv. in 1000 g . . . . .	57.1	71.1	84.3
Chlorine in precipitate answering to 1000 g mixture. mg-equiv..	1.81	1.34	0.85
Nitrogen in precipitate answering to 1000 g mixture. mg-equiv..	61.2	47.1	31.5
Cl/N in precipitate . . . . .	0.0297	0.0286	0.0270
p <sub>H</sub> in solution . . . . .	2.948	3.015	3.024
cc 0.1 n HCl added per 100 g mixture = mg-equiv. in 1000 g . . .	3	3	3
Chlorine in mixture. mg-equiv. in 1000 g . . . . .	3.76	3.71	3.71
Chlorine in solution. mg-equiv. in 1000 g . . . . .	2.39		2.51
Nitrogen in mixture. mg-equiv. in 1000 g . . . . .	120.5	120.5	119.1
Nitrogen in solution. mg-equiv. in 1000 g . . . . .	20.8	23.8	26.2
Chlorine in precipitate answering to 1000 g mixture. mg-equiv..	1.37		1.20
Nitrogen in precipitate answering to 1000 g mixture. mg-equiv..	99.7	96.7	92.9
Cl/N in precipitate . . . . .	0.0137		0.0129
p <sub>H</sub> in solution . . . . .	3.190	3.216	3.216

shaking was here 48 and 72 hours respectively (Table 2, section 1). In a similar manner, three experiments analogous with these were carried out (Table 2, section 2) the concentration of hydro-

Table 3.

Period of shaking, in hours...	24	48	24	48
cc 0.1 n HCl added per 100 g mixture = mg-equiv. i 1000 g	1.5	1.5	0.75	0.75
Chlorine in mixture. mg-equiv. in 1000 g .....	1.67	1.67	0.86	0.90
Chlorine in solution. mg-equiv. in 1000 g .....	1.36	1.40	0.78	0.83
Nitrogen in mixture. mg-equiv. in 1000 g .....	29.0	29.0	28.9	29.6
Nitrogen in solution. mg-equiv. in 1000 g .....	5.14	5.73	2.22	2.38
Chlorine in precipitate answering to 1000 g mixt. mg-equiv...	0.31	0.27	0.08	0.07
Nitrogen do. ....	23.8	23.3	26.7	27.3
Cl/N in precipitate.....	0.013	0.011	0.003	0.003
pa <sub>H</sub> in solution .....	3.109	3.095	3.353	3.338

chloric acid here being half as high. Table 3 gives the details of two other series of experiments with different quantities of casein.

In these tables, it will first be noted that the quantity of chlorine in the mixture does not coincide with the quantity of acid added. The cause of this difference is, that Suspension III contained some NaCl, which we had not washed out of the casein precipitate, for fear of not getting filterable suspensions of the casein. We afterwards found, however, that this fear was unfounded, and as it was awkward to reckon with this sodium chloride content, we made our suspensions chlorine-free. It is of no importance here.

It will further be seen that we did not succeed, in any of these experiments, in obtaining a solubility constant with regard to time (mg-equiv. N in 1000 g solution). During the first 24 hours, the increase in solubility is greatest, after that, it seems rather to become constant; i. e. the solubility increases proportionally with the time. How great it is, and how rapidly it increases, depends on the concentration of acid and the activity of hydrogen ions in the solution. The first thing that happens when the casein comes in contact with the hydrochloric acid and water

is probably that, as van Slyke and his assistants have shown, some of the hydrochloric acid is adsorbed on to the solid particles of casein. This process takes place, as we know, rapidly, and while it is going on, the concentration of hydrogen- and chlorine ions must fall to a certain value, so to speak, before the actual process of dissolution can commence. During dissolution, both casein and HCl (see table) pass over into the solution, and in the course of the first 24 hours, the activity of the hydrogen ions falls to the values noted. After that, it decreases but slowly; the solubility increases more slowly, the ratio between HCl and N in the precipitate remaining approximately constant, while the amount of chlorine in the solution rises simultaneously with the casein content.

The tables show that after 24 hours' shaking — with the same casein content — we obtain a solubility increasing with the concentration of hydrochloric acid. Apart from the fact that this solubility is quite arbitrarily chosen (after 30 hours we should get a different value) it would be interesting to ascertain whether this determination of solubility could be reproduced independently of the state in which the casein used for the experiment happens to be; whether freshly precipitated, dried etc. Kondo's work (l. c. p. 7 Tab. 3) gives us some data as to this. The following figures may be quoted:

	Unprecipitated, dried casein, Kahlbaum	Freshly precipitated, not dried casein, Kahlbaum
g Casein per 100 cc solution (Pre- cip. + solution) . . . . .	1	1
mg-equiv. HCl in 1000 cc solu- tion . . . . .	6.0	6.0
mg-equiv. Cas-N in 1000 cc solu- tion . . . . .	50.8	71.2
pH . . . . .	3.08	3.08

As was to be expected, the freshly precipitated casein dissolves much more rapidly than the dried, and, as we shall later on have occasion to note, with undried preparations it makes a considerable difference whether the casein has been just precipitated or has been left to stand, as in the preparation III actually used.

Finding it impossible rapidly to obtain equilibrium by this method, we tried another way. 40 cc of suspension III was mixed with 36 cc 0.1 n HCl and 124 cc water, and shaken for 24 hours in a thermostat. At the expiration of that time, the mixture was no longer viscous, but exhibited the character of a clear, slightly yellowish solution, which on centrifugation only yielded a very slight amount of precipitate. This solution was transferred to a collodium tube, having a glass stirrer inside, and surrounded on the outside by water which was changed four times a day. In this manner, we dialysed for a week with stirring, and this gave only a very slight precipitate, though 30 cc of the 36 cc HCl diffused out in the course of two days. At the end of the week, the solution was removed from the collodium tube and filtered and analysed. The following figures show the result:

Chlorine in the solution, mg-equivalents in 1000 g . . . . .	1.00
Nitrogen in the solution, mg-equivalents in 1000 g . . . . .	87.0
$p_{\text{aH}}$ . . . . .	3.92
0.789 g casein dissolves in 100 cc 0.001 n HCl (cf. p. 8).	

As will be seen, this method gives a far higher solubility than before, and far higher also than the values noted on pp 8, 10 and 11. But we have not yet with certainty obtained equilibrium.

We were therefore obliged to proceed as described in the following (Section 5).

#### 4. The terms employed.

In the following pages, we shall make use of the terms noted below:

1°. In mixture of precipitate and solution.

$Z_{\text{Cl}}$ : Milligramme equivalents of chlorine in 1000 cc mixture.

$Z_{\text{HCl}} = C_{\text{HCl}}$  (Precipitate + solution): Milligramme equivalents HCl in 1000 cc of mixture.

$Z_{\text{N}} = C_{\text{N}}$  (Precipitate + solution): Milligramme equivalents of N in 1000 cc mixture.

2°. In the solution.

$C_{Cl}$ : Milligramme equivalents chlorine in 1000 cc solution.

$C_{HCl}$ : Milligramme equivalents HCl in 1000 cc solution.

$C_N$ : Solubility of the casein: Milligramme equivalents of N in 1000 cc solution.

3°.

$Q_N$ :  $C_N/C_N$  (Precipitate + solution) =  $C_N/Z_N$ .

$HCl/N$ : Ratio between no. of milligramme equivalents HCl and N in the precipitate.

$c_{Cl}$  is the chloride normality in the mixture ( $= Z_{Cl}/1000$ ).

## 5. Method of attaining equilibrium and investigating the stability of the same.

We made the following experiment.. 270 cc suspension II + III (see p. 4) was mixed with 135 cc 0.1 n HCl and 270 cc water. The whole was shaken for 12 hours in a thermostat at 18°, whereby all the casein was dissolved, and we obtained a homogenous solution marked X. (28/10 1924). The solution was placed in an ice chest.

28/10. Samples were drawn off for determination of chlorine and nitrogen in X. We did not weigh these samples, but used, as in the following, standardized pipettes for taking them. The following solution Y was then prepared in a standardized measuring flask of 100 cc.

15.60 cc 0.1 n NaOH + 6 cc 1 n NaCl + water to 100 cc.

A sample of 50 cc of X was drawn off with a pipette into a medicine bottle, and to this was added, first 100 cc of water, and then 50 cc of the Y solution, carefully, down the side of the medicine bottle, so as to avoid as far as possible any mixing during the addition. When the addition was finished, the bottle was shaken suddenly and violently round. This gave a whitish precipitate in very fine particles, extremely slow to settle. After rotation in a thermostat for 2 hours at 18°, it was centrifuged at 10000 revolutions as mentioned on p. 9 and the nitrogen content (corrected for evaporation), surplus acid, hydrogen ion activity and chlorine ion activity were determined in the solution centrifuged off, which was clear, albeit with some opalescence.

The results are shown in Table 4 marked A.



<sup>30</sup>/10. 50 cc of X was drawn, precipitated with NaOH and NaCl (Y solution) in the same way as before. The whole was shaken for 15 hours and centrifuged and analysed as before. Marked *B*.

<sup>31</sup>/10. The same experiment repeated, only with 24 hours' rotation. Marked *C*.

<sup>1</sup>/11. The same, with 24 hours' rotation. Marked *D*.

Table 4.

Solution X: 182.4 mg-equiv. nitrogen in 1000 cc,  $C_N = 182.4$   
19.82 - — HCl - - -  $C_{HCl} = 19.82$

	A	B	C	D
Period of shaking, in hours..	2	15	24	24
$Z_N = C_N$ (precip. + solution)..	45.6	45.6	45.6	45.6
$Z_{Cl}$ .....	20.0	20.0	20.0	20.0
$Z_{HCl} = C_{HCl}$ (precip. + solution).	1.055	1.055	1.055	1.055
$C_N$ in solution.....	26.0	20.8	21.4	21.3
$C_{HCl}$ — .....	0.695	0.590	0.610	0.605
N in precip. mg-equiv. ....	19.6	24.8	24.2	24.3
HCl - — .....	0.360	0.465	0.445	0.450
HCl/N in princip.....	0.0184	0.0187	0.0184	0.0185
$p_{aH}$ .....	3.936	3.960	3.932	3.954
$p_{aCl}$ .....	1.821	(measured only for A).		

$p_{aH}$  of X = 2.15.

This method of determining solubility, which seems to give constant and reproducible results, is open to the following main objections. Firstly, there is the possibility that the casein is decomposed by the high concentration of hydrogen ions, in solution X, and that this decomposition is responsible for the high values for solubility thus obtained (c. f. Table 3). And secondly, it might be that we had here a kind of super-saturated solution, in labile equilibrium.

As regards the first objection, the constant solubility from day to day seems certainly to suggest that any far-reaching decomposition is out of the question. The same thing is shown by the osmotic pressures found by Kondo, which are likewise constant from day to day, as also his formol-titrations of hydrochloric acid casein solutions. We have ourselves carried out a small

series of experiments in this respect and found that the ratio between formol-titratable N and total-N in a hydrochloric casein solution with  $p_{aH} = 2.2$  altered in the course of 5 days by 10%, namely, from 0.052 to 0.058. The corresponding value for casein, which had not been subjected to the action of hydrochloric acid, was 0.052. (Titration was in both cases carried to 2. stage<sup>1</sup>) and correction made for the base-binding action of the casein itself).

In a later paper (p. 58 of this No.) one of the writers will revert to this question.

In order to meet the second of the two objections noted above, the following experiments were carried out, at a somewhat later period, and with a different preparation of casein, *B* (see p. 51).

To 100 cc of suspension *B* in a 200 cc measuring flask was added, drop by drop, and with violent shaking, 20 cc 0.1 n NaOH and water to 200 cc. This dissolved the casein, and the solution showed  $p_{aH} = 6.92$ . This stock solution was analysed for nitrogen, and the following drawn off: A) 50 cc to which was added 100 cc water and afterwards, carefully, to avoid mixing, 50 cc of the following solution:



After this had been added, the whole was suddenly and violently shaken round. The casein was thus liberated at first at the isoelectric point, but was not able to precipitate, before it was partially dissolved again in the surplus acid. The mixture thus produced was shaken for 72 hours, samples being drawn off every 24 hours, and centrifuged and analysed as above described.

B and C) 50 cc, treated in a similar manner, but with different quantities of acid and sodium chloride. The results are shown in Table 5.

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<sup>1</sup> H. Jessen Hansen: Die Formoltitration. E. Abderhalden. Biochem. Arb. meth. pag. 243.

Table 5 (Stability Experiment, 18°).

A.  $c_{Cl} = 0.01$  n.

Period of shaking, in hours ..	24	48	72
$Z_{HCl}$ : (cc 0.1 n HCl in 100 cc mixture).....	1.0	1.0	1.0
$Z_N$ : Nitrogen in mixt. mg-equiv. in 1000 cc .....	45.0	45.0	45.0
$C_N$ : Nitrogen in solution .....	30.9	30.1	30.2
$pa_H$ .....	3.891	3.886	3.882

B.  $c_{Cl} = 0.02$  n.

$Z_{HCl}$ : (cc 0.1 n HCl in 100 cc mixture).....	1.0	1.0	1.0
$Z_N$ : Nitrogen in mixture mg-equiv. in 1000 cc .....	45.0	45.0	45.0
$C_N$ : Nitrogen in solution .....	15.9	15.4	15.2
$pa_H$ .....	3.988	3.983	3.976

C.  $c_{Cl} = 0.05$  n.

$Z_{HCl}$ : (cc 0.1 n HCl in 100 cc mixture).....	1.9	1.9	1.9
$Z_N$ : Nitrogen in mixture mg-equiv. in 1000 cc .....	45.0	45.0	45.0
$C_N$ : Nitrogen in solution .....	20.7	20.5	18.1
$pa_H$ .....	3.669	3.671	3.679

This table shows that the solutions obtained in the usual way from the centrifuged mixture have a nitrogen content approximately independent of the period of shaking. The slight variation of the figures is in an opposite direction to what we should expect if there were no equilibrium, as the casein has been dissolved, and not precipitated as in the experiments described in Table 4; and save for the figure 18.1, in section 3, column 3 of Table 5, an unusually low figure here, we must, having regard to the amorphous character of the substance in question, and its possible tendency to transformation, which is perhaps not very marked, as for instance that of egg albumin (which, inter

alia, could not stand the continued shaking required for equilibrium experiments) certainly call them satisfactory.

As the values thus found for solubility agree in order of magnitude with those given by precipitation of a hydrochloric casein solution (cf. Table 5, section 2, with Table 4) the possibility of a supersaturated state of the casein in the solution is but slight. The only question now is, with what degree of accuracy the same equilibrium can be reproduced when we precipitate the casein from an acid solution with base, and when it is precipitated from an alkaline solution with a surplus of acid. It is difficult to get exactly the same point from both sides, as the method of taking samples from the casein suspension is only accurate to 5—10 % and the concentration of acid is determined as the difference between a quantity of acid and a quantity of base, one of which is often only 5—10 % greater than the other, whereby the relative measurement error is multiplied by as much as 20. For a slight error in the acid concentration will be of double significance. If there be a little too much acid, then there will not be so much precipitate formed, and as less acid will then also be bound by the precipitate, the concentration of hydrogen ions and the solubility will further increase. Similarly, again, when there is too little acid.

It is therefore necessary to determine the entire curve, or a portion of the curve for the dependence of the casein's solubility upon the activity of hydrogen ions in one of the two ways described in Tables 4 and 5, and then make separate determinations by the other method and see how they come out relatively to the curve. Though the determination of these solubility curves is actually the object of the principal section of this work, we have nevertheless preferred here to quote a single series of experiments serving to elucidate this question, which is one of great importance to the whole problem of solubility.

The casein employed was the later preparation *B* (see p. 51). The experiments noted under *A* and *B* in Table 6 were carried out precisely as described on p. 14 and p. 16. The period of shaking is noted in the table.

Table 6.

Stability experiments, 18°.  $c_{Cl} = 0.05$ 

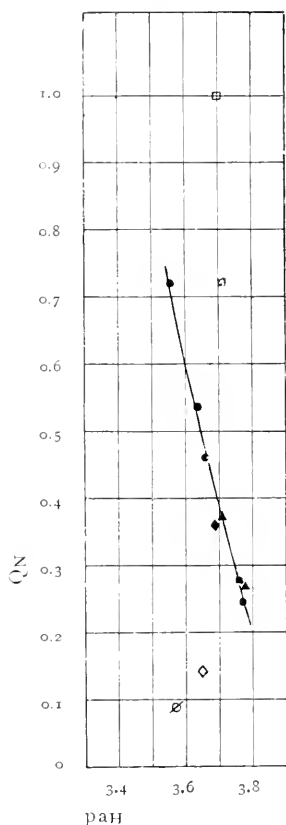
Experimental remarks.	$Z_{HCl}$	$Z_N$	$C_N$	$p_{aH}$	$Q_N$	Mark Fig. 1.
A. from the basic side:	2.10	43.0	30.9	3.551	0.719	
Rapid addition of acid. Sha-	1.90	—	23.1	3.640	0.536	
king for 24 hours. During	1.90	45.0	20.7	3.669	0.460	●
this period, no visible alte-	1.90	49.7	13.9	3.761	0.280	
ration in the appearance of	1.90	—	13.8	3.751	0.278	
the mixture .....	1.70	43.0	10.6	3.767	0.247	
B. From the acid side:	1.90	49.7	13.5	3.784	0.272	▲
Rapid addition. As A.....	1.90	—	18.8	3.719	0.378	
C. From the basic side:						
Slow addition. Two hours'						
shaking .....	1.90	45.0	6.5	3.650	0.144	◇
24 hours' shaking .....	1.90	—	16.2	3.693	0.360	◆
Great alteration in 24 hours.						
D. From the acid side. As C.						
2 hours' shaking .....	1.90	45.0	45.0	3.701	1.000	□
24 — — .....	1.90	—	32.5	3.710	0.722	■
Great alteration in 24 hours.						
E. Casein suspension B +						
HCl + NaCl. 24 hours ...	1.90	—	3.9	3.567	0.087	○

As will be seen from Table 4, first column, fifth row, equilibrium appears rather rapidly on the addition of the base; somewhat the same is the case when we approach from the basic side, and no visible alteration in the state of the mixture was discernible in the course of the 24 hours.

The concentrations given in Table 6 are expressed in mg-equivalents per litre (see also p. 13). That the 6 experiments with the concentration of hydrochloric acid at 1.90 do not give the same solubility is due to the fact that the quantity of casein was not the same in all cases, and since the precipitate binds

acid, the solubility is lowest where the quantity of casein per 1000 cc  $Z_N = C_N$  (Precipitate + solution) is greatest i. e. where there is most precipitate. For the rest, the fluctuations in the solubility are due to uncertainty in the measurement of the acid (as mentioned on p. 18) and it is therefore the relation of the

Fig. 1.



solubility to the activity of hydrogen ions, which is directly measured in the solution after attaining equilibrium, that we should particularly notice. The value  $Q_N$  in the last column of Table 6 is, as mentioned on p. 14 equal to the ratio between  $C_N$  solution and  $Z_N = C_N$  (precipitate + solution). For it was found in the main experiments, as we shall describe further later on, that it is  $Q_N$  which, at the same activity of hydrogen ions, is a constant independent of the total quantity of casein in 1000 cc, whereas  $C_N$  is approximately proportional with this quantity, i. e. with  $Z_N$ . It is therefore  $Q_N$  which we have taken as ordinate in Fig. 1, in order to compare the values obtained with different total quantities of casein.  $p_{aH}$  is the abscissa.

The determinations from the acid and from the basic side lie on the same curve, and we can therefore say, that this can be fairly accurately reproduced, even though the individual points may be difficult to determine, owing to the sources of error above noted. It is therefore probable that the state of the solution after 24 hours' shaking is, with the method here employed, of the nature of a reproducible state of equilibrium.

For further surety we tried adding the acid, or the base as the case might be, slowly, and with shaking during abt 1 minute. We then found (Table 6 section D) that in the case where we had precipitated with base (and with NaCl) — i. e. tried to establish a precipitating equilibrium, — no precipitate was formed, as we had calculated, even after 2 hours' shaking and subsequent

centrifugation. But the solution was highly opalescent. In the course of 24 hours' shaking we got a precipitate which could be centrifuged off, so that the solution must have been super-saturated to begin with, and with decreasing solubility approached the state of equilibrium characterised by our curve. This is plainly visible in Fig. 1, the square points.

In the case where we had precipitated with acid, (Table 6 section C) passing the isoelectric point, where we let the casein precipitate to the characteristic flakes and then added the surplus of acid, we had, at first, and after 2 hours' shaking, hardly anything in the solution; the precipitate was flaky, the solution clear as water, with a slightly bluish tint. In the course of 24 hours, the precipitate apparently changed character, becoming much finer in the particles, exactly like that we obtained by sudden precipitation, while the solution also took on a characteristic slight opalescence, showing that more casein had dissolved. This was also evident from the analysis. The mixture had, then, with increasing solubility, approached the state of equilibrium shown in our curve. The rhombic points in Fig. 1 show this distinctly.

There can thus be no doubt as to the reality of the equilibrium.

The last row, E, in Table 6, gives the results of an experiment in which we took the calculated quantity of the casein suspension B and added HCl to the concentration 1.90 as before and NaCl ( $c_{\text{Cl}} = 0.05$ ) and shook for 24 hours. The result, indicated in Fig. 1 by a barred circle, shows on comparison with the other experiments and with the observations in the foregoing (p. 12) how enormously the rate of solubility of the casein depends on its state or immediate history. Casein which has been precipitated out, washed and left to stand in water dissolves very slowly compared with a freshly precipitated product. and the latter mode of obtaining equilibrium is therefore as mentioned on p. 2, most inadvisable, as it would take months to complete. Indeed, with low concentrations of hydrochloric acid, it might lead to altogether erroneous results, as the alteration in solubility in course of time is so slight that it might well seem possible to obtain from day to day solubility values which were constant within the limits of experimental error. Without going further into the question as to whether the same applies to solubility

of casein in a base, we would merely mention that we have by our method, in some experiments on the basic side of the isoelectric point, obtained far higher values for solubility than those found by E. J. Cohn and J. Hendry<sup>1)</sup> by shaking casein with a base. As the difference here may possibly be due to the fact that our solutions were 0.01 n as regards NaCl, and as the circumstances necessitate a critical examination similar to that above noted in regard to the solubility in hydrochloric acid, it is too early as yet to decide the question.

It must be pointed out that in our solubility experiments, which must in several respects be called preliminary, but have nevertheless helped to elucidate certain sides of the constitution of casein, we did not attain, nor did we expect the accuracy obtainable when working with pure substances. Indeed, it is difficult, from the experience we have gained, to see any means of getting better results. On the one hand, there seems no better way than the one we have chosen, unless one is willing to undertake equilibrium experiments lasting months; on the other hand, our method has certain arbitrary features, which will be dealt with in the following.

Ordinarily speaking, the precipitation of one phase in another is the most direct method of forming a two-phase system in equilibrium. The addition of base, for instance, effected with violent shaking, is completed in a moment; a revolution takes place in the mixture, whereby, from ionised casein particles of casein- $\text{nH}^+$  or  $\text{mCl-casein-nH}^+$  ( $\text{n}$  greater than  $\text{m}$ ) there is formed uncharged casein or casein-HCl, which condenses into larger particles, appearing as a more or less pronounced precipitate in the mixture. At higher salt concentrations (0.05 n NaCl) this precipitate is of such a character that when left to stand, it settles, leaving a somewhat cloudy mixture above it, consisting of fine particles of precipitate and a slightly opalescent solution which can be obtained by centrifuging, the opalescence increasing with increasing activity of hydrogen ions, increasing casein content. The appearance of the precipitate also alters with the activity of hydrogen ions, being finest at the highest activities of hydrogen ions, where there is least of it, and assuming more of the flaky character it has at isoelectric reaction as we approach this point.

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<sup>1)</sup> Journ. Gen. Physiol 5, 5, 521 (1923).



Finally, the casein lies in the form of pure white flakes in a solution clear as water.

At lower salt concentrations, 0.01—0.02 n. the precipitate consists of smaller particles, does not settle, and gives the mixture a rather milky appearance. The precipitate centrifuged off is more sticky than at higher salt concentrations, and the solutions are more opalescent. They show a characteristic Tyndall effect, but no visible particles in the ultramicroscope. Curiously enough, the opalescence increases with decreasing activity of hydrogen ions, decreasing casein content, up to a certain point in the vicinity of isoelectric point, where the precipitate suddenly changes character, becoming flaky, while the solution at once becomes perfectly clear. It is the region between this point and the isoelectric point we endeavour to get when preparing casein by precipitating it with acid from a basic solution. This sudden change in the appearance of the solutions is not accompanied by any parallel break in the solubility curves, and we may therefore conclude that the portion of dissolved casein responsible for the opalescence noted must be small in comparison with the total quantity of casein dissolved.

This opalescence has previously been explained by the supposition that the casein really dissolved had a »schutzcolloid« effect which enabled it to keep some undissolved casein in suspension, thus producing opalescence. Incomplete in detail as this theory may be, it has doubtless a good deal of truth in it. If an uncharged particle of casein encounters on its way a charged particle, it is quite as likely that it should join this as that it should join with the uncharged particles it meets, and form precipitate with them. Now it may be so, on the one hand that associated casein ions of this type are stable in the solution, as something that »must be there«, and if the opalescence at higher salt concentrations is due to such large particles, then their formation must be reversible, as we also get opalescent solutions by dissolving flocculated casein (cf. Exp. C Table 6). In this case, we have a static condition, and the phenomenon must be investigated together with the qualities of the solution in a state of equilibrium. (It is characteristic that the opalescence disappears when we add acid to the saturated solution, which must occasion

a process of the type:  $nH^+$ -casein,  $x\text{casein} + mH^+ = nH^+$ -casein  $+ x (mH^+$ -casein)).

On the other hand, it may be that the larger charged particles formed are in irreversible suspension in the solution, and in this respect, Exp. D Table 6 affords so features of interest. The marked opalescence found in the supersaturated solution at the commencement of the experiment immediately after the slow addition of base and NaCl suggests that parts of the casein in the mixture must be present in such irreversible suspension, and in a state of equilibrium, there is really only a third of the total casein content in solution. The suspension however, is not very stable, and although the charge of the complex particles retards their condensation to larger particles, and thus delays the formation of the precipitate, we can still, after the lapse of 24 hours, centrifuge out about half the suspended portions. The question now is: Does the sudden addition of acid or base and NaCl occasion such irreversible suspension, and do we, in the state we have called equilibrium, include in the solubility such casein particles as cannot, it is true, be centrifuged off after 24 hours, but might perhaps, after standing for months, be able to condense into a precipitate, particles of the same character as those formed in Exp. B, only slower to react? The question does not affect the order of magnitude of the solubility found, because, as we have pointed out before, the suspended portion of the casein is small in proportion to the total amount of casein dissolved, and equilibrium can be established from either side; it might well however, have some effect on the accuracy of the solubility determination, especially at lower salt concentrations (see pp. 42—43). It will also be understood that there is something rather arbitrary in our method of separating the precipitate from the solution, viz. by centrifuging for 15 minutes at 10000 revolutions per minute. Fortunately, both the period of centrifuging and the number of revolutions can be varied within wide limits, without any marked effect on the result. After centrifuging for 30 minutes, for instance, at 15000 revolutions, the solubility was only 1 % lower than before, though the experiment was made with one of the most opalescent of the solutions.

We must however, doubtless reckon with an experimental error on these grounds, and here too, we have the difficult ques-

tion as to how far the solubility depends on the size of the particles in the precipitate, and similar problems. Nevertheless, the experiments described in the foregoing show that despite all fear of getting unstable conditions, it is yet possible to obtain values for solubility which are reproducible with very fair accuracy again and again. And as the problems mentioned demand a more precise analysis than we have been able to carry out within the scope of the present work, we have preferred to define the solubility as follows:

The solubility of casein in hydrochloric acid (and NaCl) is to be understood as meaning the quantity of casein (or the number of milligramme equivalents of Casein-N) which, during centrifugation for 15 minutes in a centrifuge with radius 8 cm and 10000 revolutions per minute, is not centrifuged out from a mixture consisting of two phases, each containing casein, with a total volume of 1 litre, and prepared from a clear solution of casein in HCl or NaOH by precipitation with NaOH (and NaCl) or surplus of HCl (and NaCl), the addition being made carefully, without shaking the liquids together, and the mixing effected suddenly, by violent shaking, the whole being then subjected to 24 hours' shaking in a thermostat at 18° before being centrifuged.

## 6. Main Experiments on Solubility of Casein in Hydrochloric Acid (+ NaCl).

### *a) The experimental results.*

As the method of working has been described in detail in the previous chapter, it will here suffice to give briefly the experimental results.

In all the experiments, we started from the acid side, and the casein used was mixture II + III (see p. 4). We determined the solubility at the chloride concentrations 0.01, 0.02, 0.05 and 0.1 n, and with varying concentrations of hydrochloric acid; we also used two different total concentrations of casein in the mixture (precipitate + solution)  $Z_N$ , viz. abt. 45 mg-equiv. N per 1000 cc and abt. 90 mg-equiv. N per 1000 cc mixture. One experiment is described in detail in Table 7, the results are shown in Tables 8 to 11. As to the terms employed see p. 13-14.

Table 7.

Stock Solution A: 270 cc suspension II + III, + 135 cc 0.1 n HCl + 270 cc water. Shaken for 12 hours until all was dissolved.  $\text{pH}$  2.2. 50 cc for chlorine determination, twice 5 cc to Nitrogen determ.

- 
- a). Solutions containing 10 cc suspension in 100 cc.  
 50 cc stock solution contain 20 cc suspension and 10 cc 0.1 n HCl.  
 $a_1$  50 cc stock solution + 7.4 cc 0.1 n Na OH + 1 cc 1 n NaCl  
 + water to 200 cc  
 $a_2$  50 cc stock solution + 8.0 cc 0.1 n Na OH + 1 cc 1 n NaCl  
 + water to 200 cc  
 $a_3$  50 cc stock solution + 8.6 cc 0.1 n Na OH + 1 cc 1 n NaCl  
 + water to 200 cc  
 $a_4$  50 cc stock solution + 9.2 cc 0.1 n Na OH + 1 cc 1 n NaCl  
 + water to 200 cc.

The following solutions were prepared:

$a'_1$	14.80	cc	0.1 n	Na OH	+	2 cc	1 n	NaCl	+	water to 100 cc
$a'_2$	16.00	—	—	—	+	—	—	—	—	—
$a'_3$	17.20	—	—	—	+	—	—	—	—	—
$a'_4$	18.40	—	—	—	+	—	—	—	—	—

and  $a_1$   $a_2$   $a_3$   $a_4$  were prepared from 50 cc stock solution + 100 cc water (well shaken) and 50 cc of  $a'_1$   $a'_2$   $a'_3$   $a'_4$  (violent shaking). The mixtures are then of the following composition:

$a_1$	abt 125	mg	cas.-N	+	2.6	cc	0.1 n	HCl	+	1.74	cc	1 n	NaCl	in 200 cc
$a_2$	—	—	—	+	2.0	—	—	—	+	1.80	—	—	—	—
$a_3$	—	—	—	+	1.4	—	—	—	+	1.86	—	—	—	—
$a_4$	—	—	—	+	0.8	—	—	—	+	1.92	—	—	—	—
$c_{Cl}$	constant = 0.01 n. $Z_{Cl} = 10$ .													

The solutions were shaken in medicine bottles in a thermostat at 18° for 24 hours. Centrifuged for 15 min. at 10000 revolutions per minute (weighed before and after). Analysed for nitrogen (corrected for evaporation), for surplus acid (do. do.) and activity of hydrogen ions and of chlorine ions measured.

The experiments described in the following tables were carried out in a similar manner.

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Table 8. Total chloride concentration  $c_{Cl} = 0.01 \text{ n}$ ;  $Z_{Cl} = 10.0$ .

$Z_{Cl} = C_{Cl}$ (Prec. + Sol.).....	10.00	10.00	10.00	10.00
$C_{Cl}$ in solution .....	9.92	9.68	9.74	9.75
HCl in precipitate .....	0.08	0.32	0.26	0.25
$Z_{HCl} = C_{HCl}$ (Prec. + Sol.).....	1.230	0.930	0.630	0.330
$C_{HCl}$ in solution .....	[1.150]	0.610	0.370	0.080
HCl in precipitate.....	[0.080]	0.320	0.260	0.250
$Z_N = C_N$ (Prec. + Sol.) .....	45.3	45.3	45.3	45.3
$C_N$ in solution.....	33.8	23.8	15.6	1.2
N in precipitate .....	11.5	21.5	29.7	44.1
HCl/N in precipitate .....	[0.007]	0.015	0.009	0.006
$Q_N$ .....	0.746	0.525	0.344	0.026
$pa_H$ .....	3.721	3.899	4.035	4.286
$pa_{Cl}$ .....	2.121	2.112	2.101	2.092
$pa_H + pa_{Cl}$ .....	5.842	6.011	6.136	6.378
$Z_{Cl} = C_{Cl}$ (Prec. + Sol.).....	10.00	10.00	10.00	10.00
$C_{Cl}$ in solution.....	9.84	9.84	9.46	9.50
HCl in precipitate .....	0.16	0.16	0.54	0.50
$Z_{HCl} = C_{HCl}$ (Prec. + Sol.).....	2.365	1.865	1.265	0.665
$C_{HCl}$ in solution.....	2.210	1.705	0.730	0.170
HCl in precipitate .....	0.155	0.160	0.535	0.495
$Z_N = C_N$ (Prec. + Sol.) .....	90.7	90.7	90.7	90.7
$C_N$ in solution.....	82.6	70.1	34.5	4.7
N in precipitate .....	8.1	20.6	56.2	86.0
HCl/N in precipitate .....	0.019	0.008	0.010	0.006
$Q_N$ .....	0.911	0.773	0.380	0.052
$pa_H$ .....	3.721	3.789	3.984	4.217
$pa_{Cl}$ .....	2.166	2.172	2.154	2.133
$pa_H + pa_{Cl}$ .....	5.887	5.961	6.138	6.350

Table 9. Total chloride concentration  $c_{\text{Cl}} = 0.02 \text{ n}$ ;  $Z_{\text{Cl}} = 20.0$ .

$Z_{\text{Cl}} = C_{\text{Cl}}$ (Prec. + Sol.) . . . . .	20.00	20.00	20.00	20.00
$C_{\text{Cl}}$ in solution . . . . .	19.39	19.55	19.51	19.67
HCl in precipitate . . . . .	0.61	0.45	0.49	0.33
$Z_{\text{HCl}} = C_{\text{HCl}}$ (Prec. + Sol.) . . . . .	1.355	1.055	0.755	0.350
$C_{\text{HCl}}$ in solution . . . . .	0.750	0.605	0.265	0.020
HCl in precipitate . . . . .	0.605	0.450	0.490	0.330
$Z_{\text{N}} = C_{\text{N}}$ (Prec. + Sol.) . . . . .	45.6	45.6	45.6	45.6
$C_{\text{N}}$ in solution . . . . .	28.1	21.3	8.4	0.4
N in precipitate . . . . .	17.5	24.3	37.2	45.2
HCl/N in precipitate . . . . .	0.035	0.018	0.013	0.007
$Q_{\text{N}}$ . . . . .	0.616	0.467	0.184	0.009
$\text{p}a_{\text{H}}$ . . . . .	3.880	3.954	4.078	4.504
$\text{p}a_{\text{Cl}}$ . . . . .	1.821	1.821	1.805	1.805
$\text{p}a_{\text{H}} + \text{p}a_{\text{Cl}}$ . . . . .	5.701	5.775	5.883	6.309
$Z_{\text{Cl}} = C_{\text{Cl}}$ (Prec. + Sol.) . . . . .	20.00	20.00	20.00	—
$C_{\text{Cl}}$ in solution . . . . .	19.67	18.75	18.99	—
HCl in precipitate . . . . .	0.33	1.25	1.01	—
$Z_{\text{HCl}} = C_{\text{HCl}}$ (Prec. + Sol.) . . . . .	2.610	2.110	1.510	—
$C_{\text{HCl}}$ in solution . . . . .	2.285	0.865	0.500	—
HCl in precipitate . . . . .	0.325	1.245	1.010	—
$Z_{\text{N}} = C_{\text{N}}$ in solution . . . . .	91.2	91.2	91.2	—
$C_{\text{N}}$ in solution . . . . .	78.3	37.8	17.8	—
N in precipitate . . . . .	12.9	53.4	73.4	—
HCl/N in precipitate . . . . .	0.025	0.023	0.014	—
$Q_{\text{N}}$ . . . . .	0.859	0.414	0.195	—
$\text{p}a_{\text{H}}$ . . . . .	3.712	3.983	4.038	—
$\text{p}a_{\text{Cl}}$ . . . . .	1.834	1.840	1.854	—
$\text{p}a_{\text{H}} + \text{p}a_{\text{Cl}}$ . . . . .	5.546	5.823	5.892	—

Table 10. Total chloride concentration  $c_{Cl} = 0.05 \text{ n}$ ;  $Z_{Cl} = 50.0$ .

$Z_{Cl} = C_{Cl}$ (Prec. + Sol.).....	50.00	50.00	50.00*	50.00
$C_{Cl}$ in solution.....	49.69	49.11	48.91	49.15
HCl in precipitate.....	0.31	0.89	1.09	0.85
$Z_{HCl} = C_{HCl}$ (Prec. + Sol.)....	2.435	1.935	1.435	0.935
$C_{HCl}$ in solution.....	2.130	1.045	0.345	0.090
HCl in precipitate.....	0.305	0.890	1.090	0.845
$Z_N = C_N$ (Prec. + Sol.).....	46.0	46.0	46.0	46.0
$C_N$ in solution.....	37.1	19.9	5.4	0.9
N in precipitate.....	8.9	26.1	40.6	45.1
HCl/N in precipitate.....	0.034	0.034	0.027	0.019
$Q_N$ .....	0.807	0.433	0.117	0.020
$p a_H$ .....	3.447	3.666	3.886	4.168
$p a_{Cl}$ .....	1.463	1.451	1.468	1.459
$p a_H + p a_{Cl}$ .....	4.910	5.117	5.354	5.627

$Z_{Cl} = C_{Cl}$ (Prec. + Sol.).....	50.00	50.00	50.00*	50.00
$C_{Cl}$ in solution.....	49.68	49.15	48.08	48.26
HCl in precipitate.....	0.32	0.85	1.92	1.74
$Z_{HCl} = C_{HCl}$ (Prec. + Sol.)....	4.370	3.620	2.870	1.870
$C_{HCl}$ in solution.....	4.055	2.770	0.955	0.130
HCl in precipitate.....	0.315	0.850	1.915	1.740
$Z_N = C_N$ (Prec. + Sol.).....	91.9	91.9	91.9	91.9
$C_N$ in solution.....	80.8	63.6	20.3	1.3
N in precipitate.....	11.1	28.3	71.6	90.6
HCl/N in precipitate.....	0.028	0.030	0.027	0.019
$Q_N$ .....	0.879	0.692	0.221	0.014
$p a_H$ .....	3.449	3.608	3.801	4.121
$p a_{Cl}$ .....	1.470	1.485	1.476	1.480
$p a_H + p a_{Cl}$ .....	4.919	5.093	5.277	5.601

Table II. Total chloride concentration  $c_{Cl} = 0.10n$ ;  $Z_{Cl} = 100.0$ .

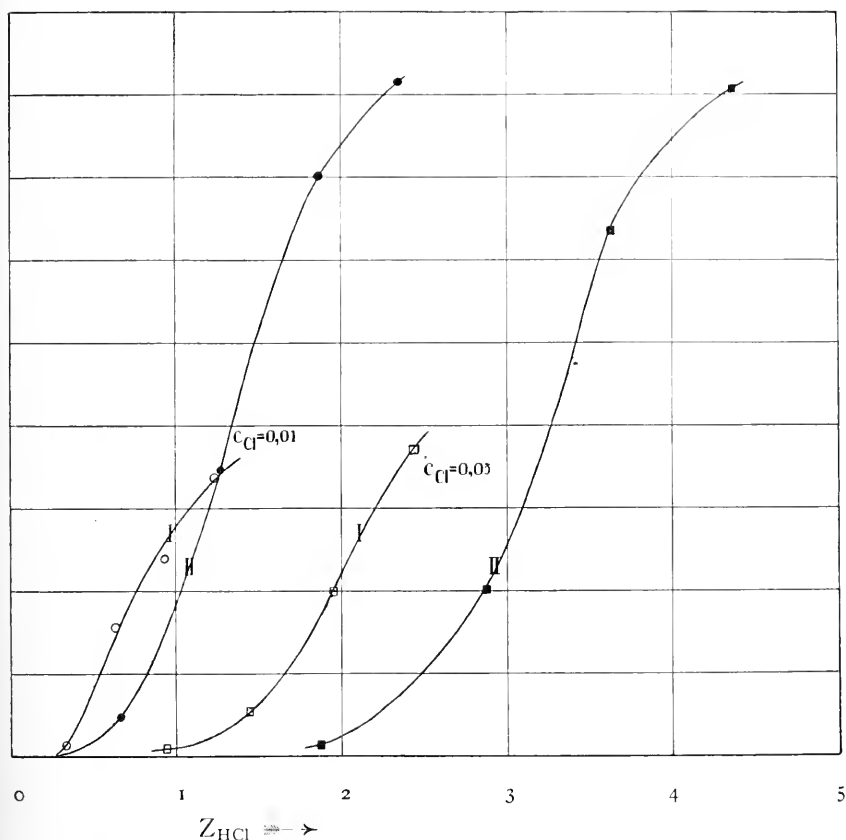
$Z_{Cl} = C_{Cl}$ (Prec. + Sol.).....	100.00	100.00	100.00	100.00
$C_{Cl}$ in solution.....	98.30	97.98	98.18	98.60
HCl in precipitate.....	1.70	2.02	1.82	1.40
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$H_{HCl} = C_{HCl}$ (Prec. + Sol.)....	3.200	2.700	2.200	1.500
$C_{HCl}$ in solution.....	1.500	0.685	0.380	0.100
HCl in precipitate.....	1.700	2.015	1.820	1.400
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$Z_N = C_N$ (Prec. + Sol.).....	44.6	44.6	44.6	44.6
$C_N$ in solution.....	12.2	4.9	1.7	0.9
N in precipitate.....	32.4	39.7	42.9	43.7
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HCl/N in precipitate.....	0.052	0.051	0.042	0.032
$Q_N$ .....	0.274	0.110	0.038	0.020
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$p_{aH}$ .....	3.210	3.404	3.591	4.019
$p_{aCl}$ .....	1.199	1.203	1.205	1.203
$p_{aH} + p_{aCl}$ .....	4.409	4.607	4.796	5.222
<hr/>				
$Z_{Cl} = C_{Cl}$ (Prec. + Sol.).....	100.00	100.00	100.00	100.00
$C_{Cl}$ in solution.....	97.60	96.87	96.17	96.54
HCl in precipitate.....	2.40	3.13	3.83	3.46
<hr/>				
$Z_{HCl} = C_{HCl}$ (Prec. + Sol.)....	6.400	5.800	4.800	3.800
$C_{HCl}$ in solution.....	4.000	2.675	0.970	0.340
HCl in precipitate.....	2.400	3.125	3.830	3.460
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$Z_N = C_N$ (Prec. + Sol.).....	89.2	89.2	89.2	89.2
$C_N$ in solution.....	43.5	30.5	8.3	1.8
N in precipitate.....	45.7	58.7	80.9	87.4
<hr/>				
HCl/N in precipitate.....	0.053	0.053	0.047	0.040
$Q_N$ .....	0.488	0.342	0.093	0.020
<hr/>				
$p_{aH}$ .....	2.991	3.161	3.406	3.669
$p_{aCl}$ .....	1.207	1.215	1.205	1.205
$p_{aH} + p_{aCl}$ .....	4.198	4.376	4.611	4.874



*b) Casein regarded as a mixture.*

We will first consider the hydrochloric acid binding of the precipitate and the circumstances attending on the same. Fig. 2 shows graphically the relation between  $C_N$ , the solubility, and  $Z_{HCl}$ , the acid concentration in the mixture, at two chloride con-

Fig. 2.



centrations  $c_{Cl}$  0.01 and 0.05 n. The solubility  $C_N$  is the ordinate, and  $Z_{HCl}$  the abscissa. A phenomenon of the same character as described by Kondo (see p. 1) is at once discernible in these curves. The solubility at the same total concentration of acid,  $Z_{HCl}$ , decreases as the amount of precipitate increases, curves I here representing experiments with  $Z_N$  abt 45, and those marked II experiments with  $Z_N$  abt 90.

This however, evidently only applies when the concentration

of hydrochloric acid is not too high, i. e. as long as there is still a considerable quantity of precipitate, as in this case the amount of acid bound will be great and very dependent on  $Z_N$ . At higher concentrations of hydrochloric acid, where the solubility of the casein is higher, the quantity of precipitate decreases, and therefore also the quantity of acid bound; curve I approaches Curve II — at  $c_{Cl} = 0.01$  they intersect — and the relation between solubility and amount of precipitate therefore seems from this figure to be different when we can disregard the amount of acid bound. It is therefore of interest to ascertain how matters stand when we take the solubility together with the actual concentration of hydrochloric acid,  $C_{HCl}$ , as determined by titration in the saturated solution. And on comparing Fig. 2 with Fig. 3, where  $C_N$  is ordinate and  $C_{HCl}$  abscissa, it will be seen that the effect observed by Kondo, due to binding of hydrochloric acid by the precipitate, completely covers the following characteristic phenomenon: that the solubility at the same true hydrochloric acid concentration,  $C_{HCl}$  increases, on the contrary, with the quantity of precipitate, with  $Z_N$ .

Similar phenomena have been observed before, and have given rise to various explanations. One thing, however, is certain; a result such as the foregoing suggests that the precipitate we have in the mixture is not constant in its composition. The determination of solubility is therefore, as emphatically pointed out by Sorensen<sup>1)</sup> in »Studies on Proteins« and also in the works noted on p. 2 one of the best means of ascertaining whether a substance — in this case a protein — consists of one sort of molecule complexes or molecules, or not.

By this means, Sorensen and his assistants succeeded in throwing light on the constitution of the globulins. Among other works, we may mention that quoted on p. 22, by Cohn and Hendry, in which the determination of solubility is used as a criterion of the purity of the casein. That these investigators came to the conclusion that casein is a homogeneous substance, while we ourselves arrived at the opposite result, is a point we shall have occasion to refer to in a later work.

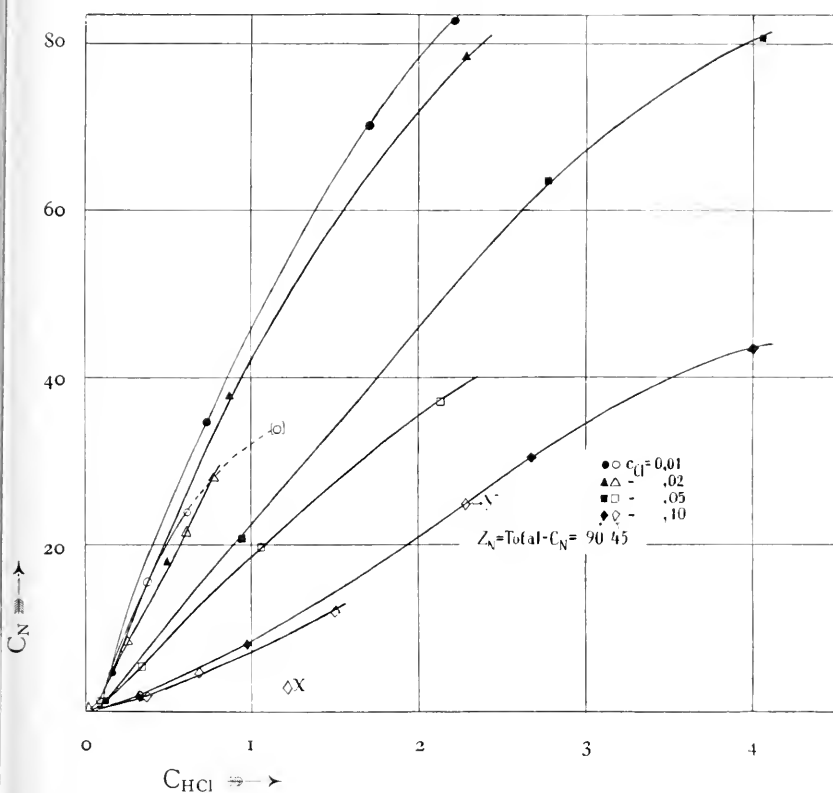
In our own case, we know, of course, that the precipitate

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<sup>1</sup> Comptes-rendus du Lab. Carlsberg. 12 (1917).

is for one reason at least, variable in its composition, to wit, on account of the quantity of acid bound. In order to illustrate this point we will first of all consider a simple example. Let us imagine a weak base, B, of slight solubility, whose hydrochloric salt, HBCl is also sparingly soluble. If we have both

Fig. 3.



the base and the chloride in a solid form, in contact with their saturated aqueous solution, we must then have:

$$\frac{a_{BH^+}}{a_H} = k_1 \text{ and } a_{BH^+} \cdot a_{Cl} = k_2, a_H \cdot a_{Cl} = K,$$

where  $a_{BH^+}$  is the activity of the monovalent kation,  $a_H$  and  $a_{Cl}$  the activity of the hydrogen and chlorine ions respectively. If HCl, water and B are the only three components, and if, as assumed, the base and the chloride at the bottom form each its own phase, then according to Gibbs' phase rule, the system

must be entirely determined by temperature alone. The addition of an arbitrary quantity of HCl would, it is true, alter the proportion between chloride and base in the precipitate to the advantage of the chloride, but the composition of the solution must remain the same until all the base has been transformed into chloride or dissolved. On adding a fourth component, NaCl, the concentration of this last may be arbitrarily chosen, but once it is chosen, the whole of the system is definitely fixed.

It is quite a different matter if the salt and the base only form one phase on the bottom, if they form a solid solution. Calling the ratio between no. of molecules M of the two components in the precipitate  $y$ , we can then write

$$\frac{a_{BH^+}}{a_H} = F(y) \cdot \frac{1}{1+y} \text{ and } a_{BH^+} \cdot a_{Cl} = F_1(y) \cdot \frac{y}{1+y}$$

$$a_H \cdot a_{Cl} = F_2(y) \cdot y. \quad (1)$$

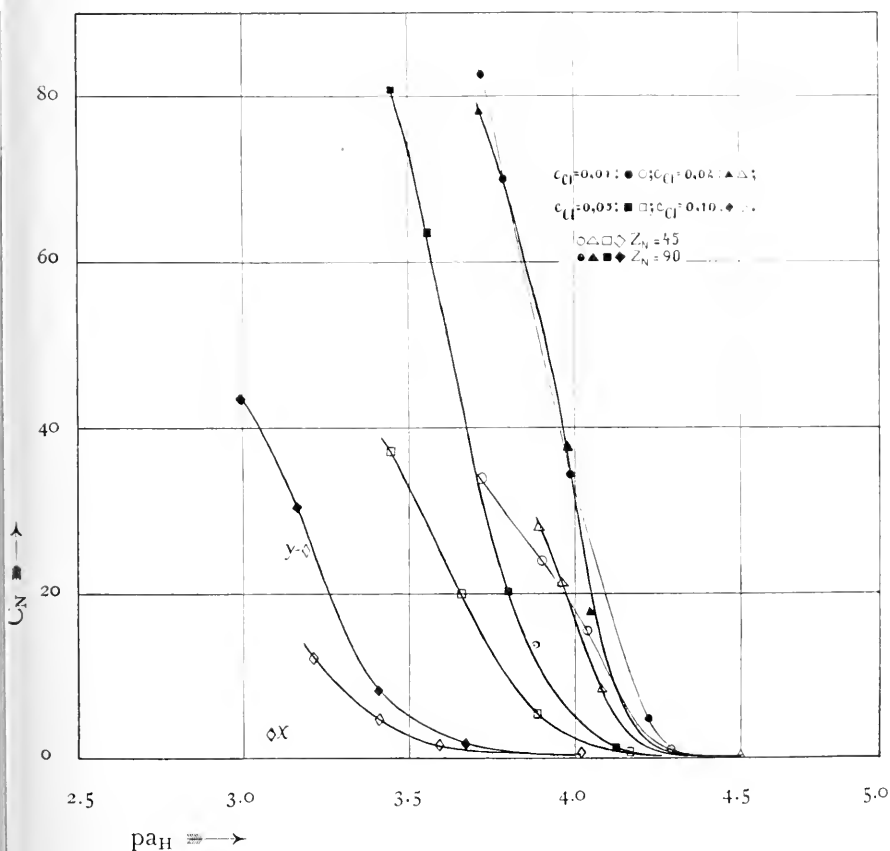
where  $y$  is  $M_{HCl} / M_B$  in the precipitate.

If the precipitate were an ideal mixture, the functions  $F(y)$ ,  $F_1(y)$  and  $F_2(y) = F_1(y)/F(y)$  would be constants. But in a case like this, which has been so little investigated, we can do no more than point out that as expressions of the forces acting between molecules in the precipitate they are functions solely of the nature of the substance and of  $y$ , so that  $y$ , according to (1) is determined exclusively by the product of chlorine ion and hydrogen ion activity in the solution. According to the phase rule we can here, where there is a phase less, for four components besides the temperature, choose two of the concentrations in the solution at will, e. g. the hydrochloric acid and sodium chloride concentrations. But once they have been fixed, the internal state of the solution is also determined, and the solubility, for instance, is independent of the ratio between the amounts of precipitate and solution. We can also choose the activity of hydrogen ions and chlorine ions as independent variables, or the chloride concentration and the hydrogen ion activity, and express the position thus: Given the same chloride concentration and the same hydrogen ion activity, the solubility must also be the same, irrespective of any solid solution which may be formed by the components B and HCl. That this is not the case with casein, is shown by Fig. 4, where  $pa_H$

( $= -\log a_H$ ) is the abscissa, solubility the ordinate, and the point indication the same as on Fig. 3.

The position here is so pronounced that  $C_N$ , at the same  $pa_H$ , is approximately proportional with  $Z_N$ , so that the values

Fig. 4.

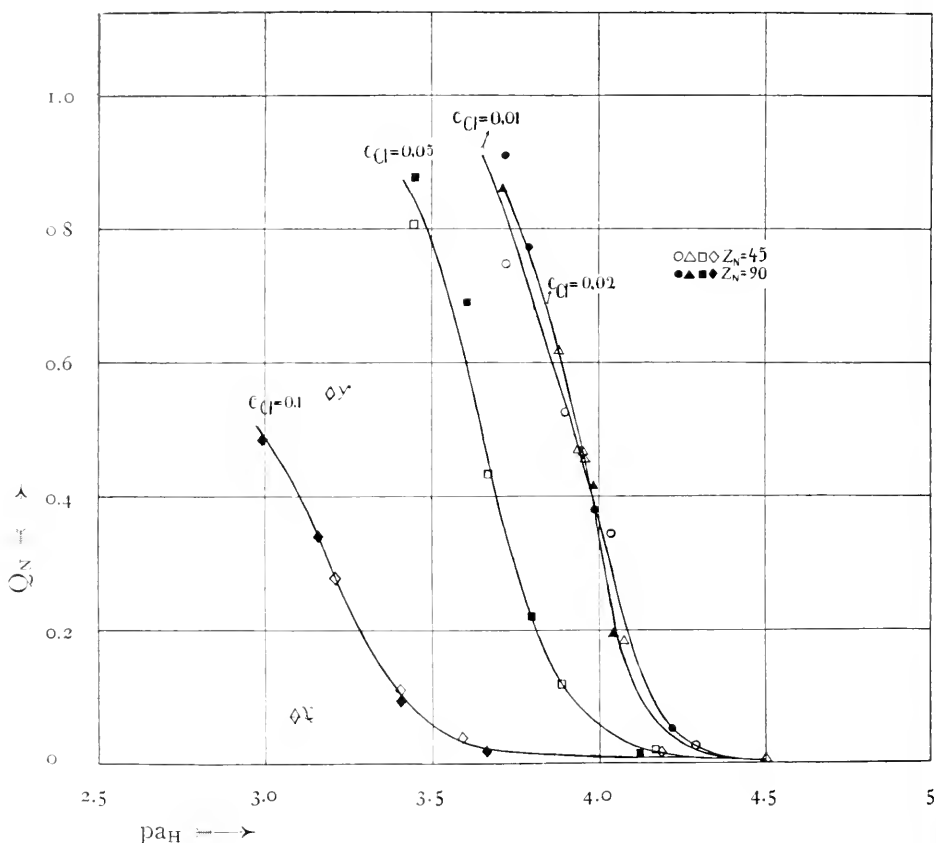


for  $Q_N = C_N / Z_N$  set off as ordinates in Fig. 5 lie very nearly in the same curve, answering to the same chloride concentration,  $c_{Cl}$ .

We must here point out however, that the concentration of chloride in the solution (see Tables 8—11) is not precisely 0.01, 0.02 etc. ( $C_{Cl} = 10, 20, 50, 100$ ) but somewhat less, and therefore not always exactly alike when  $Z_N$  is 45 and when it is 90, at the same  $pa_H$ . The differences, however, are very slight. An

attempt at correction, from our knowledge of the dependence of solubility on the concentration of the salt when  $\text{pa}_\text{H}$  is kept constant, gave the following results for the experiments marked \* in Table 10.

Fig. 5.



	found	calculated	found	calculated
$\text{pa}_\text{H}$ .....	3.886	(3.886)	3.801	(3.801)
$C_\text{Cl}$ .....	48.91	50.0	48.08	50.0
$C_\text{N}$ .....	5.4	<b>4.9</b>	20.3	<b>19.2</b>

As these are the two most pronounced experiments we have therefore disregarded the differences.

After this, the only way in which we can explain the remarkable behaviour of the casein is by supposing that the system contains more components than the four: casein, water,

NaCl and HCl; or in other words, that casein is a mixture of two or more substances, at any rate, the casein we were using.

And now as regards further examination of the solubility of casein, the peculiarities found are so pronounced (see Fig. 3 and 4) that it is necessary first of all to form some idea of their causes before proceeding to consider the results which were expected, namely, that the solubility increased with the activity of hydrogen ions, owing to the increasing ionisation, and decreased with increasing concentration of chlorine ions owing to the fact that chlorine ions were bound to the protein molecules. The first step towards elucidation of the mentioned phenomena — the dependence of the solubility on the quantity of precipitate — has, it is true, been made by Sørensen (l. c.) but we are still far from attaining a full quantitative comprehension of the position.

On applying Gibbs' phase rule to the system: casein, HCl, NaCl, water, we are led to the supposition that the number of components must exceed 4, or more properly, it must be more than 1 in excess of the number of phases. It must however be emphasised — as on p. 24 — that the system here considered is of such a kind as to render doubtful any application of the phase rule which is not based upon deeper theoretical considerations. And this for four reasons. Firstly, because the precipitate is amorphous, secondly, because the particles of the precipitate are small, and thirdly because the circumstances connected with solid solutions of several components are altogether of a complex nature. On the other hand, it will be seen that the high velocity of precipitation we have used affords the best guarantee for equilibrium between solution and precipitate if the latter is a solid mixture of several protein components (see later on), and the possibility of establishing the equilibrium from both sides has given us the conviction that it is permissible to apply the phase rule to our case in the same way as we have done in our simple examples. The results of our fractionating experiments afford a basis for this view.

The case, however, is very complicated and we will therefore here content ourselves with the following quite practical and preparatory treatment of the question, at the same time however, referring to the observations on p. 46 and hoping also to revert later on to the theoretical side of the matter.

As will be seen from Fig. 4, the solubility is, with a fairly high degree of approximation, proportional, at the same activity of hydrogen and chlorine ions, with  $Z_N$ , the total nitrogen content in 1000 cc of mixture. We shall not come to the explanation of this remarkable phenomenon presently; for the moment, all we have to do is to ascertain whether the casein present in the solution in the two cases,  $Z_N = 45$  and  $Z_N = 90$  may not be different. This we will do by means of the acid-binding capacity (cf. Sorensen l. c. p. 32) or the mean valency (cf. Linderstrom-Lang<sup>1</sup>).

If we call the concentration of a protein solution  $c_k$ , the mean valency  $n_m$  we obtain, for the relation between a quantity of acid added,  $y$  (expressed as concentration in the solution) and the concentration of hydrogen ions  $c_H$ , the following equation:

$$y = c_H - c_H^0 + c_k \cdot n_m, \quad (4)$$

where  $c_H^0$  is the concentration of hydrogen ions at isoelectric point. Disregarding this as slight in comparison with  $y$ , we obtain:

$$n_m = \frac{y - c_H}{c_k} = \frac{y - c_H}{K \cdot C_N}, \quad (5)$$

and  $n_m$  should now, at the same activity of hydrogen ions and concentration of salt, be independent of the casein concentration, as found by Sorensen for egg albumin, provided the casein were alike. As will be seen from Table 12, we have carried out the calculation for the two salt concentrations 0.05 and 0.1, taking  $a_H = c_H$  (the error thus committed tends in the opposite direction to the divergencies found). The results are shown

Table 12.

$Z_N$	$Z_{Cl}$	$C_N$	$c_{HCl} = y$	$y - c_H$	$K \cdot n_m = \frac{y - c_H}{C_N}$	$p_{aH}$
45	50	37.1	$2.130 \cdot 10^{-3}$	$1.773 \cdot 10^{-3}$	$0.478 \cdot 10^{-4}$	3.447
—	—	19.9	1.045 —	0.829 —	0.417 —	3.666
90	—	80.8	4.055 —	3.700 —	0.458 —	3.449
—	—	63.6	2.770 —	2.523 —	0.397 —	3.608
—	—	20.3	0.955 —	0.797 —	0.392 —	3.801
45	100	12.2	1.500 —	0.883 —	0.723 —	3.210
90	—	43.5	4.000 —	2.980 —	0.685 —	2.991
—	—	30.5	2.675 —	1.985 —	0.651 —	3.161

<sup>1</sup>, Comptes-rendus du Lab. Carlsberg, 15, 7 (1924).

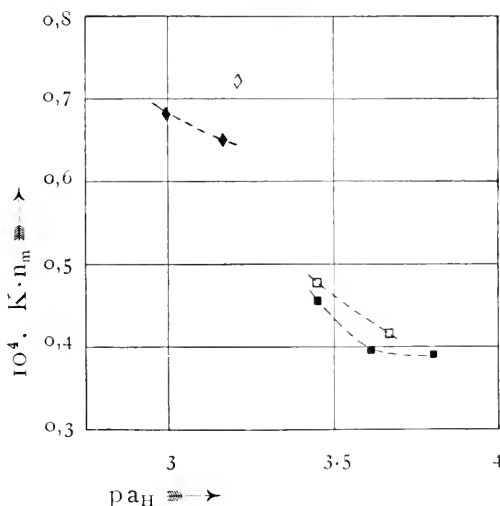


graphically in Fig. 6, where  $pa_{II}$  is the abscissa and  $K \cdot n_m$  (see equation 5) the ordinate. The notation of points is the same as in Fig. 3.

From this it appears that there is a slight but distinct difference in the mean valency of the casein in the two cases, ( $Z_N = 45$  and  $Z_N = 90$ ).

We then proceeded to fractionate the casein, considering that by separating the precipitate from the solution in a solubility experiment, and cleansing the casein in each separately, we should, on renewed solubility determination, find the more easily soluble components of the casein in the solution and the less soluble portions in the precipitate. It is naturally to be expected that the solubility of the hypothetical components

Fig. 6.



of the casein should, a priori, be different, as otherwise, the phenomena we have observed would not have occurred at all.

50 cc of mixture II + III was mixed with 25 cc 0.1N HCl and 50 cc water, and shaken for 18 hours. With this solution we then repeated the experiment in Table II, section 2, column I, the dissolved and the precipitated casein being here present in approximately equal quantities.  $Z_N = 90$ ,  $Z_{HCl} = 6.4$ ,  $c_{Cl} = 0.10$ . The precipitate was dissolved in HCl, after being centrifuged off, precipitated at the isoelectric point and washed free of chlorine with water. Dissolved in water and 12.5 cc 0.1N HCl and diluted to 100 in a measuring flask. Stock solution marked X. As regards the solution, this was precipitated to the isoelectric point, the casein washed free of chlorine, and dissolved in the same way as the precipitate. Stock solution Y.

With these two stock solutions, we carried out two solubility experiments, entirely alike, but yielding

nevertheless widely dissimilar results, as will be seen from Table 13. Figs. 3, 4 and 5, where the determinations are set out graphically, together with the others, marked X and Y, answering to precipitate or solution, show even more distinctly that the casein from the precipitate is far less soluble, that from the solution far more easily soluble than the unfractionated casein.

Table 13.  $c_{Cl} = 0.1$ ,  $Z_{Cl} = 100$ .

$Z_N$	$Z_{HCl}$	$C_N$	$C_{HCl}$	N Prec.	HCl Prec.	HCl/N Prec.	$p_{aH}$	$Q_N$	$p_{aH} +$ $p_{aCl}$
X, Precipitate									
42.2	3.410	3.0	1.210	39.2	2.200	0.056	3.083	0.072	4.28
Y, Solution									
45.2	3.380	25.2	2.280	20.0	1.100	0.055	3.194	0.558	4.39

*c) On the character of the binding of hydrochloric acid by the precipitate.*

We will now, in conclusion, consider the binding of hydrochloric acid by the precipitate.

We shall not here proceed on the lines indicated on p. 34 regarding the binding as a formation of chloride in solid solution in the casein, though the functional relation between  $y$  ( $M_{BHCl}/M_B$ ) and  $a_H \cdot a_{Cl}$  in the simple case might perhaps invite further investigation, but will regard the binding as a kind of adsorption, at the same time however, emphatically pointing out that since the precipitate is formed suddenly in the mixture, the binding must also be supposed to take place inside the particles of the precipitate, so that we should perhaps rather call it an absorption. We will therefore also point out that the marked binding must be due, not so much to a great surface of the particles of the precipitate as to a characteristic feature in casein (and many other proteins) namely, its marked capacity to combine, chemically or physically, with HCl, which is also responsible for the binding of chlorine ions in a casein solution (cf. Kondo l.c.).

Using the activity term introduced by Lewis, then for any precipitate capable of combining with HCl, and in equilibrium

with any solution, containing hydrogen ions and chlorine ions, the state of equilibrium will be expressed by:

$$a_H \cdot a_{Cl} = k \cdot a_{HCl}^s \quad (6)$$

where  $a^s$  ( $s$  is not an exponent) is the activity of HCl in the adsorption zone.  $a^s$  is determined by the quantity,  $M^s$ , of the acid in the adsorption zone, by the nature of the adsorbent substance, by other adsorbed substances present; in a word, by the forces acting in the area under consideration. In a simple case like that investigated by van Slyke and his assistants, where finely pulverised casein was mixed in the cold with dilute hydrochloric acid, without any noteworthy quantity of casein being dissolved, the matter is comparatively easy, as  $a^s$  becomes a function of  $M^s$  and the nature of the casein alone, so that with a constant surface area per unit of weight of casein,  $A^s$ , the quantity of HCl adsorbed per weight unit of casein, becomes a function of  $a_H \cdot a_{Cl}$  alone.

$$A^s = f(a_H \cdot a_{Cl}) \text{ at constant temperature.}$$

In our case, where we can, at the same activity of hydrogen and chlorine ions, have widely different quantities of nitrogen in solution, it will be interesting now to investigate whether the amount of hydrochloric acid bound is here also a function of  $a_H \cdot a_{Cl}$  alone and if so, what, as we could, by such investigation, perhaps obtain information as to any alterations in the state of the solid phase, surface, etc. We have therefore, in Fig. 7 set off as ordinate the values for bound hydrochloric acid in proportion to the amount of nitrogen in the precipitate (Cl/N) arrived at by determination with all four concentrations of salt, 0.1, 0.05, 0.02 and 0.01, and with  $Z_N = 45$  and 90. For the abscissa we have used  $p_{a_H} + p_{a_{Cl}} = -\log(a_H \cdot a_{Cl})$ . If now all these points fall in one and the same curve, it is a proof that the quantity of acid bound is a function of  $a_H \cdot a_{Cl}$  alone, irrespective of the fact that we can, for the same value of this product, have widely different concentrations of hydrogen ions, chlorine ions and casein.

It will be noticed that the points here fall very nearly in the same curve. The points in brackets (point notation the same as in Fig. 3) answer to experiments where the quantity of precipitate was very slight, and the amount of hydrochloric acid

bound,  $= Z_{\text{HCl}} - C_{\text{HCl}}$  is badly determined. For the rest, systematic divergencies of any great extent are only observed in the two lowest concentrations of salt, where the quantity of acid bound at the highest concentrations of hydrogen ions, where the concentration of casein is greatest, is greater than it should be according to the common curve. As, however, the precipitate here, as pointed out on p. 23 is in finer particles, has a different appearance, is of a different state than at the lower concentrations of hydrogen ions, where the casein assumes the peculiar flaky appearance, and at the higher concentrations of salt 0.5 and 0.1, the explanation must be sought either in the increased surface area of the precipitate due to the fineness of the particles, or, as the fineness must be due to some state quality in the precipitate, to a combined alteration in the surface and in the field of force on the surface of the precipitate particles compared with the same qualities in the precipitates whose binding of HCl is represented by the common curve. As to the cause of these phenomena we will not here attempt to form any theory, and as the results of the solubility determinations at these two low salt concentrations 0.01 and 0.02 n also show characteristic divergencies (see Fig. 4) we will postpone the problem for systematic investigation later on.

The presence of the common curve, however, shows that the state of the precipitate and the size of the particles at the same  $a_{\text{H}} \cdot a_{\text{Cl}}$  is very nearly independent of the other qualities of the mixture:

$$A^s = f(a^s) = f(a_{\text{H}} \cdot a_{\text{Cl}})$$

As seen from the foregoing however, we are obliged to assume that the precipitate at the same  $a_{\text{H}} \cdot a_{\text{Cl}}$  must be of different composition at different salt concentrations and the different casein preparations, X and Y (see Fig. 7); that it is of different chemical character, and we are therefore led to the idea that it is not so much the specifically chemical character of the molecules in the precipitate, but rather certain physical qualities in their structure which determines the binding of hydrochloric acid. The experiments however, are not sufficiently accurate to permit of any conclusions in this respect, and it is possible that accurate experiments may confirm the rule very slightly indicated in Fig. 7, that the points from experiments

where  $Z_N$  is 45 lie on the whole higher than those from experiments where  $Z_N$  is 90. In these preliminary investigations, the

Fig. 7.

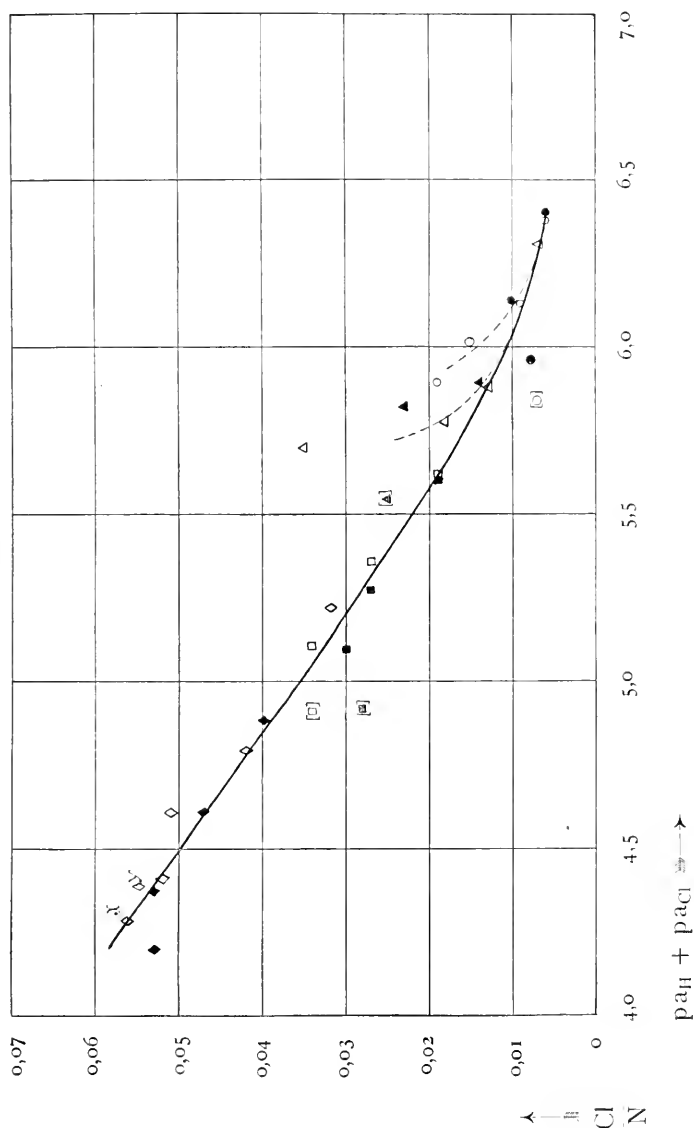


figure serves to emphasise that we do not find great alterations of the solid phase in its physical state, which is of importance for the observations in the foregoing.

As regards the actual shape of the curve, the left portion is very nearly a straight line, and  $\text{Cl}/N$  is expressed by:

$$\text{Cl}/N = 0.175 - 0.028 (\text{p}a_{\text{H}} + \text{p}a_{\text{Cl}}) \quad (7)$$

or transformed

$$\text{Cl}/N = -K \log \frac{P}{a_{\text{H}} \cdot a_{\text{Cl}}} = -0.028 \cdot \log \frac{5.6 \cdot 10^{-7}}{a_{\text{H}} \cdot a_{\text{Cl}}}$$

— an equation of the same form as those used by one of us (l. c. p. 38) in a previous work in dealing with ion adsorption, and which can be arrived at — with a certain approximation — from statistical considerations.

## 7. Survey of the Results obtained.

The results of the foregoing may be briefly summed up as follows:

1. The solubility of casein in hydrochloric acid and NaCl increases with the activity of hydrogen ions and decreases with increasing concentration of chlorine ions. (Figs. 4, 5).

2. When a hydrochloric solution of casein is precipitated with NaOH and NaCl, the precipitate binds part of the surplus acid, so that for the same total hydrochloric acid concentration in the mixture of precipitate and solution, the solubility  $C_N$  of the casein generally decreases with increasing total quantity of casein in the mixture ( $Z_N$ ), increasing quantity of precipitate and thus increasing quantity of bound acid (Fig. 2).

3. If however we determine by titration the hydrochloric acid concentration of the solution saturated with casein, we get the opposite result, as the solubility for the same true concentration of hydrochloric acid in the solution increases with increasing total casein content ( $Z_N$ ) (Fig. 3).

4. At the same activity of hydrogen ions and same concentration of chlorine ions, the solubility is likewise dependent on  $Z_N$  and approximately proportional with the same (Fig. 4 and 5).

5. The binding of hydrochloric acid by the precipitate may be regarded as a kind of adsorption on the particles of the precipitate, and is, save for the most concentrated casein solutions at salt concentrations 0.01 and 0.02 n, approximately a function of the product of hydrogen ion and chlorine ion activity alone, and a function having the form of an ordinary adsorption isotherm.

6. The phenomena mentioned under 3 and 4 can be explained by supposing that the casein we have employed consists of several different substances with different degrees of solubility, and that it forms, together with water, HCl and NaCl, a system in which the number of phases is at the outside the number of components minus 2. It has also been found that the casein remaining in the solution on precipitation when  $Z_N$  is 45 and when it is 90 is different as regards the mean valency. We also succeeded by fractionating the casein, in separating it into two groups, one more, the other less soluble than the unfractionated casein.

7. As the properties of casein are more or less analogous to those of the globulins, and as the globulin investigations carried out at the Carlsberg Laboratory have in many respects been of great importance to the planning and execution of the present researches, we will in conclusion add a few words on the solubility of the globulins as compared with what we have found in regard to casein.

In contrast to casein, the serum globulin has previously been regarded as a mixture, but the division into two parts, euglobulin and pseudoglobulin, did not prove sufficient to explain the solubility conditions, unless it were supposed that these two substances, both in precipitate and in solution, entered into combination with each other, forming complexes of varying composition, which Sorensen<sup>1)</sup> characterises by the formula  $E_p P_q$  (E, euglobulin, P, pseudoglobulin) and varying solubility, which complex-formation renders them very difficult to separate one from the other.

A sample of euglobulin-pseudoglobulin shows the following characteristic qualities:

- 1) Its solubility depends on the concentration of the neutral salt.
- 2) Its solubility is dependent on the quantity of precipitate or the total content of globulin in precipitate + solution.
- 3) The globulin in the solution is always more soluble and that in the precipitate less soluble than the original sample. (Other conditions being equal).

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<sup>1)</sup> Comptes-rend. du Lab. Carlsberg **15**, 10 (1925). Journ. Amer. Chem. Soc. **47**, 457 (1925).

4) By repeated washing of the sample with water or neutral salt solution of a certain concentration, in which it is not completely soluble, it is possible to render the precipitate as sparingly soluble in water or salt solution as desired, and it will further approach more and more towards a solubility independent of its quantity. But in solubility experiments with a neutral salt solution of higher concentration than that used for the washing, we again find the phenomenon noted under 2), and the solubility will be lower, the more concentrated the neutral salt used for washing.

From this it will be seen that the position is entirely analogous with that of casein, merely substituting »hydrochloric acid« for »neutral salt«.

The solubility experiments were as a rule carried out by first dissolving the globulin in a stronger neutral salt solution and then precipitating with water. Sorensen therefore explains the phenomenon in the following manner: At the high salt concentration, we have the complexes  $E_p P_q$  which are easily soluble at this concentration. On diluting with water to the salt concentration  $x$ , these complexes are dissociated into others rich in pseudoglobulin or rich in euglobulin, the latter in the main precipitating out, as they are less soluble, whereas the former remain chiefly in the solution, being easily soluble. This explains the difference in solubility of the globulin in the solution as compared with that in the precipitate. On washing the precipitate with more salt solution (conc.  $x$ ) some globulin rich in pseudoglobulin can be further extracted, but only to a certain limit, at which the solubility becomes very slight. If however, we use a salt solution stronger than  $x$ , then there takes place in the precipitate a liberation, or »peeling off« of complexes relatively rich in pseudoglobulin, easily soluble in this salt solution, and therefore removable by washing, but again only up to a certain limit etc. The dependence of the solubility on the quantity of precipitate is explained by the fact that some of the complexes are almost entirely soluble, so that their concentration must fall to one-half when the solution is diluted to double.

Apart from the fact that the solubility anomalies are still more pronounced in the casein, where there even seems to be a tendency on the part of the solubility to increase to more than double when  $Z_N$  is doubled, (though this may be due to ex-



perimental error<sup>1)</sup>) this view nevertheless gives a good illustration of the position as regards casein. We must however, here surely suppose that there are more than two components, as Sørensen also points out in the case of the globulins, but the theoretical difficulties of the problem are so great that it is too early yet to express any definite opinion.

Generally speaking however, we may say this much: that casein, like globulin, is a mixture of two or more components associated by forces of some kind both in the precipitate and in the solution. We can separate the different groups of components or complexes by addition of hydrochloric acid and fractionate them by fractionated precipitation, and it is to be expected that the lower the concentration of hydrochloric acid chosen, and the less the amount then dissolved, the more will the dissolved groups of components diverge from the original casein in solubility, being more and more easily soluble. We shall in the next paper consider other qualities parallel with the solubility.

As our casein was a trade product, it must of course be primarily the task of subsequent investigation to determine whether the qualities exhibited by the casein were not due to the method of manufacture, or whether the same phenomena as here observed can be reproduced with other casein preparations.

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<sup>1)</sup> See p. 36.

## II. IS CASEIN A HOMOGENEOUS SUBSTANCE?

BY

K. LINDERSTRØM-LANG.

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In a previous work<sup>1)</sup> the writer has, together with S. Kodama, studied the solubility of casein in hydrochloric acid. The results there obtained however, call for confirmation and further consideration in several respects.

In the first place, the casein used for the previous experiments was prepared after the Hammarsten method, treated with alcohol and ether and dried, and there was the possibility — as already noted in the mentioned paper, p. 47 — that these processes might have brought about a decomposition, so that the casein employed would be itself a mixture of decomposition products. I have therefore prepared the casein for the present work myself, and in the most careful manner possible.

Secondly, further investigation of the separate casein fractions and the demonstration of characteristic chemical differences between them would be of the greatest interest, if it could be taken for granted that the differences in these fractions were not due to decomposition products irreversibly formed in the casein used. Though there is little reason to believe that the casein previously employed did contain such decomposition products, or that the method adopted (see p. 5) furthered the formation of such, I have nevertheless, to be on the safe side, avoided as far as possible any exposure of the casein to hydrogen ion concentrations exceeding  $\text{p}a_{\text{H}} = 4$ , or less than answering to 7. So also, in solubility determinations and fractionation, I have always proceeded from the basic side (see p. 16).

I therefore consider that the casein used in the present ex-

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<sup>1)</sup> This issue. p. I.

periments must be considered as produced, purified, and experimentally treated in the most careful manner, and since it behaved exactly as did the casein previously employed, there can be no doubt but that casein consists of two or more different molecule species, which can, by fractionation, be partly separated one from another, owing to their different solubility.

The correctness of this conclusion is confirmed by the fact that the different casein fractions not only exhibit different degrees of solubility, but differ also in phosphorus content, and in their behaviour towards calcium chloride.

That casein cannot be regarded as consisting of one species of protein molecules loosely combined with phosphoric acid, is shown by a brief series of experiments as to the solubility of casein in phosphoric acid and sodium phosphate.

### 1. Preparation of the Casein.

18 litres of unpasteurised, fresh skimmed milk was precipitated with abt. 6 litres 0.05 n HCl, which was introduced in a fine jet with violent stirring in the course of six hours. The casein liberated was filtered off after the expiration of one day on clean dusters, and washed three times with ice-cold toluol-saturated water. The first lot of water came away clear, the second and third however, cloudy and milky, probably with fats. The total volume of the rinsing water was  $3 \cdot 20 = 60$  litres. All the processes, here as in the following, were carried out as far as possible at ice-chest temperature, and with abundant toluol. The hydrogen ion concentration of the milk after the addition of acid and of the water lay between  $\text{p}_{\text{aH}} = 4.6$  and 5.

The casein thus washed was mixed with water and dissolved in portions, with violent stirring, 0.02 n NaOH being added a drop at a time. The  $\text{p}_{\text{aH}}$  of the solution, which was very milky and cloudy but did not contain undissolved casein particles, never exceeded 7, and lay between this value and 6.7, the normal concentration of hydrogen ions for milk. To the milky solution was added sodium chloride to the concentration 0.5 n, which yielded no precipitate, and the four times 6 litres of solution were shaken with 4 times 3 litres of ether, frequently and powerfully, in 10 litre flasks. In the course of 24 hours, the water

phase separated, on quietly standing, from the ether phase, which was highly viscous and blistered, whereas the water phase was fine and clear, slightly yellow in transmitted light, but the reflected light had a faint bluish tinge. The water phase was drawn off by a siphon (abt. 21 litres in all, 3 litres remaining in the ether phase or were lost by the incomplete drawing off) and shaken once more with ether (10 litres) in the same way, then centrifuged in a milk separator at 8000 revolutions per minute, volume of the centrifuge 1 litre, velocity of centrifugation 2 litres per hour.

From the last ether phase, 2 litres of ether was distilled off, the remainder being abt. 0.1 cc of a yellow, stinking liquid, which unfortunately exploded violently at  $100^{\circ}$  so that I was unable to weigh it; it doubtless consisted of ethyl-peroxide, an impurity in the ether employed. As the casein content of the 18 litres of solution drawn off from the last ether treatment was, according to the nitrogen determination, 410 g, and as the total quantity of ether only contained 0.5 g of residue at the outside, the treatment with ether in this way was evidently effective as regards the removal of fat.

The perfectly clear centrifuged solution (18 litres) was slowly precipitated once more with 0.02 N HCl, and the mixture, which contained a great deal of ether, and presumably therefore was slow to precipitate, was finally regulated to  $p_{aH} = 4.2$ , when the casein at the high salt concentration (0.5) collected in a nicely flocculated form, leaving a filtrate clear as water above it. The precipitate was filtered off on dusters, mixed with water once more, but would not settle properly again, as the quantity of acid bound by the precipitate at the high concentration of salt in the first filtrate was taken up by the less saline second lot of water, together with a quantity of casein in colloidal form. The mixture was therefore regulated again by addition of base to  $p_{aH} = 4.3$ , at which concentration of hydrogen ions the casein precipitated nicely. It was then filtered again, and water added (12 litres) after which the casein was preserved in this state for 14 days.

At the expiration of this time, the mixture was filtered. The filtrate was very slightly opalescent; its salt concentration was abt. 0.02 N and hydrogen ion concentration answering to  $p_{aH} = 4.1$ ,

higher than before. probably because more HCl had found its way from the precipitate into the solution.

On adding NaOH to  $p_{aH} = 4.8$ , the filtrate yielded a small precipitate which was rinsed with water and preserved. Mark Z (see later on).

The main precipitate was mixed with water and regulated to  $p_{aH} = 4.6$  (12 litres). The mixture was divided into two lots.

A) 3 litres. This was filtered, and water added. As the casein now settled without any regulation of the hydrogen ion concentration, it was washed four times with 4 times 1500 cc water. Finally, water was added to 2 litres. This suspension was chlorine free, and had  $p_{aH} = 4.7$ ; as stock suspension A it was used for solubility determinations and fractionation.

Suspension A. 513 mg casein-N in 100 cc mixture. 10.3 g casein-N in all.

B) The main portion, 9 litres, was dissolved again in NaOH as already described, precipitated with HCl and washed free of chlorine, until the last filtrate had  $p_{aH} = 4.8$ .

Stock suspension B. 490 mg casein-N in 100 cc mixture. 29.4 g casein-N in all.

Precipitate Z (see above) 0.195 g casein-N in all.

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A was further fractionated as follows:

250 cc stock suspension was carefully dissolved in 50 cc 0.1 n NaOH and 200 cc water;  $p_{aH} = 6.8$ . To this solution, in a 2 litre flask, was then added 89.4 cc 0.1 n HCl, 91.06 cc 1 n NaCl and water to 2000 cc, whereby part of the casein was liberated, and the mixture was left to stand with frequent shaking for 24 hours. Using the terms employed in the previous paper (l. c. p. 13) we had here  $Z_N = \text{abt. } 45$ ,  $Z_{HCl} = 1.97$ , and  $p_{aH}$  in the solution centrifuged off after 24 hours was 3.70.

1) The solution was precipitated with base to isoelectric point and the precipitate rinsed with water till it was free of chlorine,

Casein D, 0.288 g casein-N in all.

2) The precipitate was mixed with water, and base added to isoelectric point, then washed with water until the precipitate was free from chlorine.

Casein E, 0.626 g Casein-N in all.

As the quantity of casein prior to fractionation was  $2.5 \times 0.513 = 1.283$  g casein-N, 0.369 must have been lost in the rinsing. This is of importance in the following (see p. 57).

## 2. Solubility experiments with stock suspension of casein A, B, with casein D and E and with precipitate Z.

With the casein preparations thus obtained, a series of solubility experiments were carried out, chiefly in the manner previously described (l. c. p. 14—16) the equilibrium being always established from the basic side, save for a few control determinations made from the acid side of isoelectric point for testing purposes. The salt concentration  $c_{Cl} = 0.05$  n was chiefly used, and only a few measurements at 0.02 and 0.01 were made. I determined only the solubility and activity of hydrogen ions after 24 hours' shaking in a thermostat at  $18^\circ$ , and afterwards centrifuging for 15 minutes at 10000 revolutions per minute. Correction was made for evaporation during centrifugation.

The results are given in the following tables, and graphically in Fig. 1, where  $pa_H$  is the abscissa and  $Q_N$  the ordinate; the dotted curves are those from the solubility experiments in the work carried out with S. Kodama (Fig. 5, p. 36). Several of the experiments will also be known from the investigation of equilibrium p. 17 and p. 19.

It will be seen from these tables and curves, as clearly as could be wished, that the phenomena found in a previous work by Dr. Kodama and myself are also characteristic of the new casein. The solubility increases proportionately with  $Z_N$ , the  $Q_N$  values lying very nearly in the same curve. Also, as before, it is possible to separate the different components in the casein by fractionation, the easily soluble parts being found chiefly in the solution, the less soluble in the precipitate. Furthermore, it will be seen from a glance at Fig. 1 that the new casein, both A and B, neither of which has been fractionated, shows very much the same solubility as II + III (see p. 4) though it must be noted that there is a quite considerable difference in the lowest solubilities, the lowest hydrogen ion activities. And this is precisely what we should expect, for repeated washing would naturally remove chiefly the more easily soluble component groups.

Table 1. Casein A.  $c_{Cl} = 0.05$ .

Mark on Fig. 1	$Z_N$	$Z_{HCl}$	$C_N$	$p_{aH}$	$Q_N$
From basic side	44.9	2.30	35.2	3.482	0.784
□	46.4	1.94	23.9	3.667	0.515
	44.9	1.60	4.7	3.827	0.105
	44.9	1.20	1.7	4.035	0.038
■	92.8	3.20	26.4	3.742	0.284
	89.8	3.80	68.5	3.574	0.763
	89.8	2.60	5.0	3.915	0.056
From acid side					
∅	35.2	2.10	31.5	3.468	0.895
	35.2	1.80	18.9	3.601	0.537

Table 2. Casein B.

Mrk. on Fig. 1	$c_{Cl}$	$Z_N$	$Z_{HCl}$	$C_N$	$p_{aH}$	$Q_N$
△	0.01	45.0	1.0	30.9	3.891	0.687
△	0.02	45.0	1.0	15.9	3.988	0.353
Basic side	0.05	43.0	2.1	30.9	3.551	0.719
	—	43.0	1.9	23.1	3.640	0.536
	—	45.0	1.9	20.7	3.669	0.460
△	—	49.7	1.9	13.9	3.761	0.280
	—	49.7	1.9	13.8	3.761	0.278
	—	43.0	1.7	10.6	3.767	0.247
acid side	—	49.7	1.9	13.5	3.784	0.272
△	—	49.7	1.9	18.8	3.719	0.378

Table 3. Casein D.  $c_{Cl} = 0.05$ 

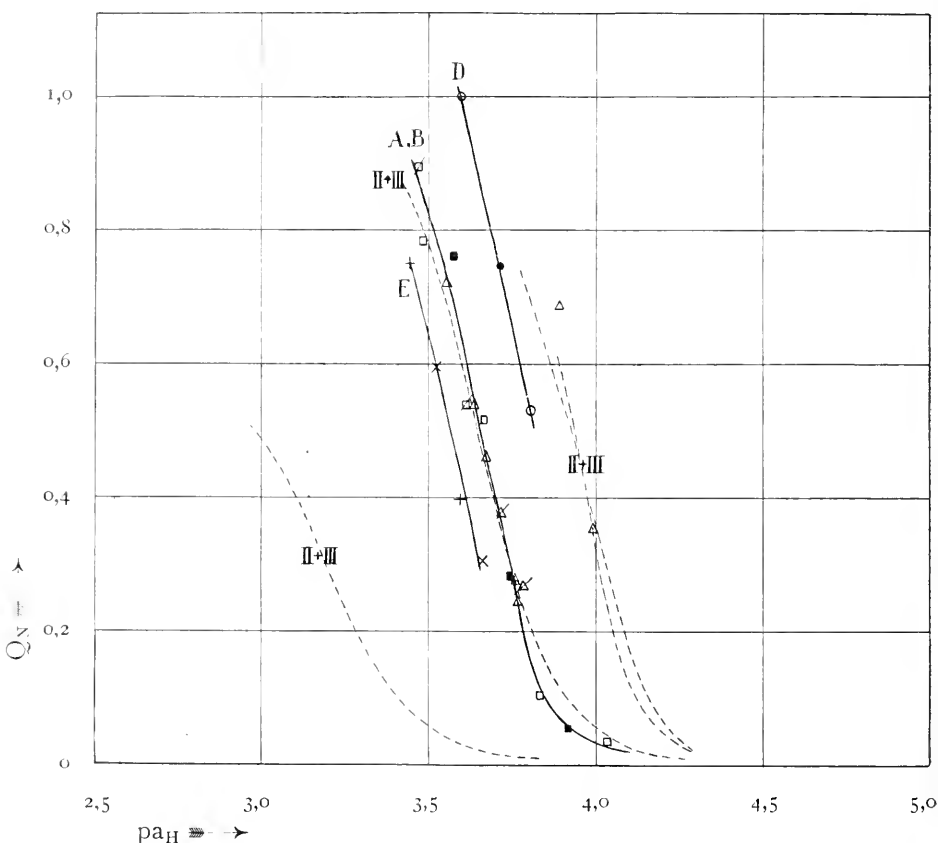
Mark on Fig. 1	$Z_N$	$Z_{HCl}$	$C_N$	$p_{aH}$	$Q_N$
Basic side.	34.3	1.9	34.3	3.586	1.000
○	34.3	1.5	18.2	3.802	0.531
●	68.6	3.0	51.3	3.709	0.748

Table 4. Casein E.  $c_{Cl} = 0.05$ 

	$Z_N$	$Z_{HCl}$	$C_N$	$p_{aH}$	$Q_N$
Basic side	44.6	2.30	33.5	3.443	0.751
+	44.6	1.94	17.7	3.598	0.397
×	89.3	3.80	53.4	3.525	0.598
	89.3	3.20	27.3	3.671	0.306

and we have here, at a certain stage, removed relatively quite a large portion of the most easily soluble, namely as Precipitate Z (see p. 51); Z being the part that would dissolve in abt. 14 days at a hydrogen ion activity answering to  $\text{pa}_\text{H} = 4$ . And now, what about the solubility of Z?

Fig. 1.



Having used the greater part of Z for chemical investigations, we were not able to make a real solubility experiment like those made with the other fractions, but qualitative experiments have shown that it is very easily soluble indeed, and has, moreover, quite unlike ordinary casein, the quality of being completely soluble in 0.2 n NaCl at isoelectric reaction (abt. 10 mg casein-N dissolved in 10 cc 0.2 n NaCl solution). Nor had it the flaky character of ordinary casein, but was of a spongy, slightly greenish appearance, and in basic solu-



tion —  $\text{pH} = 6,8$  — showed, as we shall later refer to again, no trace of cloudiness or opalescence with calcium or barium chloride. It is not a case of a trace of the lactoglobulin demonstrated by Sebelien<sup>1)</sup> here carried away by the casein, as will be shown by the following chemical investigations.

### 3. Chemical investigations of the separate fractions and casein preparations.

#### *a) Rennet reaction and behaviour towards calcium chloride.*

One of the most characteristic features about casein is its power of coagulating with rennet in a solution with hydrogen ion concentration answering to  $\text{pH} = \text{abt. } 7$ , and containing calcium salts.<sup>2)</sup> I have therefore, at the instigation of cand. polyt. H. Jessen-Hansen and Prof. Sorensen, examined the various casein preparations in regard to this quality. I proceeded as follows:

From a solution of casein in NaOH with hydrogen ion activity  $\text{pH} = 6.8$  and containing *abt. 250 mg casein-N in 100 cc*, two samples were taken, each of 5 cc, and to each was added 8 drops of a 0.6*n*  $\text{CaCl}_2$  solution. Both were heated to *abt. 38°*, and to one of them was then added 5 drops of a diluted rennet solution, to the other 5 drops of the same rennet solution after the latter had boiled for some minutes. We will call the first sample *L*, the second *K*. The result of the experiments was as follows:

Casein A.	L	K
Before heating	milky	milky
During heating	very milky	very milky
Abt $\frac{1}{2}$ minute after		
Rennet addition	completely coagul.	unaltered

Casein B (as Casein A).

Casein, nach Hammarsten (As Casein A).

Casein D.	L	K
Before heating	somewhat opalescent	somewhat opalescent
During heating	milky, but transparent	milky, but transparent
Abt. $\frac{1}{2}$ min. after addn.		
of rennet	completely coagul.	unaltered

<sup>1)</sup> Overs. Vidensk. Selsk. Forh. 1885, 1.

<sup>2)</sup> Hammarsten: Lehrbuch der physiologischen Chemie. (1910). pag. 613.

Casein E.	L	K
Before heating	very milky, opaque	very milky, opaque
During heating	apparently unaltered	apparently unaltered
Abt. $1\frac{1}{2}$ min. after addn. of rennet	completely coagul.	unaltered
Precipitate Z.		
Before heating	perfectly clear	perfectly clear
During heating	do.	do.
On adding rennet	After half a minute cloudy, then more and more opalescent at last distinctly coagulated, though apparently less com- pletely than the others	unaltered

A solution of serum-euglobulin, rich in phosphorus, at the same hydrogen ion concentration, showed no indication of coagulation.

It appears from these experiments that all the casein fractions showed the characteristic casein reaction. Particularly noteworthy is the difference in the colloidal precipitation with calcium salts exhibited by the different fractions; it looks as if D did not precipitate so much, or rather, did not give so much cloudiness as E. while Z does not cloud at all. We cannot then but ask whether it might not be possible to get a casein fraction so sparingly soluble that it yielded real precipitate with calcium salts, without addition of rennet, at the concentrations we have employed. Altogether, it seems as if possibilities were opening for getting further into the problem of rennet action, by means of these fractionating experiments with casein. I will not go into this question, however, but merely observe that I shall keep this point in view in further investigations. The experiments also serve to show that it cannot be lactoglobulin we have in the precipitate Z. Unfortunately, Sebelien<sup>1)</sup> does not give the power of rennet coagulation for lactoglobulin, but whether it

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<sup>1)</sup> l. c.

coagulates or not, it makes no difference to us here, for if it does not coagulate, then Z at any rate cannot be lactoglobulin, and if it does coagulate, then it seems reasonable to include it in the casein, since a division after such a reaction is always preferable to the usual very vague one after precipitation with salts. The point of resemblance between Z and the globulins — solubility in dilute salt solutions — seems also to be the only one, as Z for instance on heating shows entirely the characteristic casein conditions, as at  $p_H = 4.8$ , in a solution of sodium acetate-acetic acid, we found at  $65^\circ$  an opalescent cloudiness beginning from the upper portion of the liquid, and disappearing on cooling. Even when heated to  $100^\circ$  for several minutes, when the cloudiness gave place to a transparent precipitate evidently consisting of drops, considerable quantities of this precipitate were dissolved again on cooling. No globulin ever behaved like this.

*b) Phosphorus determination.*

The following phosphorus determinations were carried out with great care by Mrs. M. Sørensen, to whom I beg to express my sincere thanks. The results here likewise show peculiar points of difference between the casein preparations. The figures refer to the ratio between phosphorus and nitrogen, both in mg.

Casein Hammarsten....	P/N = 0.0498
Casein A .....	P/N = 0.0501
Casein D .....	P/N = 0.0489
Casein E .....	P/N = 0.0542
Casein Z .....	P/N = 0.0194

From this it appears that the solubility increases with decreasing phosphorus content — the first direct indication that the different components of which we imagine the casein to consist are chemically different individuals. Particular interest attaches to the low value shown by Z, since this lies below the phosphorus content even of euglobulin.

As regards D and E, there is a distinct difference between the two, though D does contain more phosphorus than we should at first expect to find; and moreover, the sum of the phosphorus contents in D and E together is not equal to that of the unfractionated casein, as  $2/3 \cdot 0.0542 + 1/3 \cdot 0.0489$  equals 0.0525 (see p. 51)

i. e. greater than 0.0501. In the precipitation and washing of the fractions, however, it is the most soluble components, those poorest in phosphorus, which would be carried off, so the result is easily understood (see p. 52).

*c) Determination of formol-titratable nitrogen.*

Formol titration is an excellent means of ascertaining whether irreversible decomposition of the casein takes place on fractionation, as an incipient hydrolysis should make itself apparent by an increase in the number of free, formol-titratable amino-groups. As already mentioned in the previous paper (p. 16) the alteration of formol-titratable nitrogen in a solution of casein at  $p_H = \text{abt. } 2$  in the course of 5 days was not considerable, and we could not therefore expect any powerful reaction in such a determination from the separate fractions as here in question. It is therefore quite natural that the experiments should give negative results, as shown in Table 5, though of course it might be possible that the different fractions contain different quantities of formol-titratable nitrogen; D for instance more, E less than the original casein, so that on being mixed together, they should give the original value. The experiments are, however, not sufficiently accurate to show this. Titration was carried to the first stage (H. Jessen-Hansen l. c. p. 16) and second stage.

Table 5.

Casein A	1. Stage	2. Stage
Formoltitr. N / Total-N .....	0.0496	0.0513
Casein D		
Formoltitr. N / Total-N .....	0.0532	0.0591
Casein E		
Formoltitr. N / Total-N .....	0.0487	0.0526

These determinations support the view that we are not dealing with irreversible transformations.

*d) General remarks on the external qualities of the different casein preparations.*

As mentioned, (p. 54) the precipitate Z differed greatly in appearance from the other casein preparations, but there was also a considerable difference between D and E in respect of colour and consistency, D being slightly greenish-blue, and looser than E, which has a greyish brown tinge.

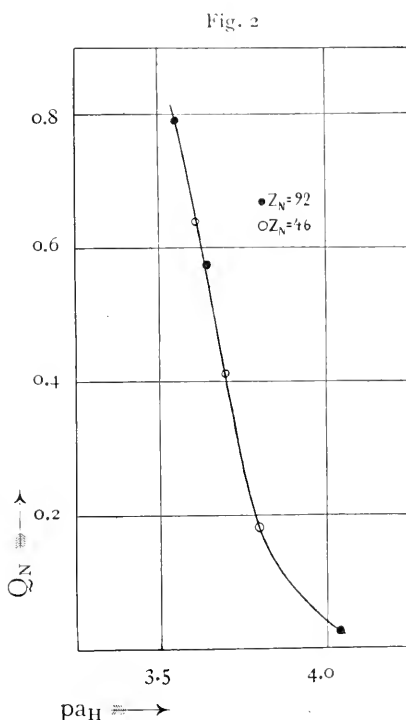
#### 4. Solubility of Casein in Phosphoric Acid (+ Sodium phosphate.)

Although it might seem improbable from the first that the two components of which at least the casein must consist (see p. 36) could be phosphoric acid and one protein, an investigation of this point may nevertheless perhaps be of some interest in view of the dependence of the solubility of the different casein fractions on their phosphorus content.

The investigation can be easily carried out; all that is needed is to keep the phosphate ion concentration or "phosphate" concentration constant in the solution. If by this means we obtain, at the same activity of hydrogen ions, a casein solubility independent of the quantity of precipitate or of  $Z_N$ , then we can regard casein as a loose chemical compound of phosphoric acid and a protein appearing as a well-defined chemical individual. If not; if we again

find, under the circumstances noted, that the solubility is a function of  $Z_N$ , then we can conclude from this that the phenomenon is at any rate not only due to phosphorus present in the casein as loosely bound phosphoric acid, and that therefore the decomposition of the casein into several groups of components must be due to causes deeper down in the protein molecule<sup>1</sup>).

In the experiment here described, I proceeded entirely as set forth in Studies on Casein I p. 16 adding phosphoric acid and sodium phosphate to a solution of casein in NaOH. At the hy-



<sup>1</sup>) The phosphorus might, for instance, be present in the form of lecithin (cf. H. Chick, Biochem. Journ. **8**, 404, (1914)) or some other organic substance containing phosphorus.

drogen ion concentrations at which the solubility was determined,  $p_{\text{aH}} = 3.5-4$  i, the phosphoric acid is exclusively monobasic, and we have therefore in the solution a mixture of  $\text{H}_3\text{PO}_4$  and  $\text{NaH}_2\text{PO}_4$ , in exactly the same way as with  $\text{HCl}$  and  $\text{NaCl}$ . The results are shown in Table 6. The concentrations noted (for the terms see p. 13 of this issue) of phosphoric acid and "phosphate" are expressed by the number of milligramme-atoms of phosphorus per litre.

$C_{\text{phosphate}}$  is determined by direct phosphorus determination in the saturated casein solution. The total phosphate concentration (precipitate + solution) was 0.05 molar answering to  $Z_{\text{phosphate}} = 50$ .

Table 6.  $Z_{\text{phosphate}} = 50.0$   $T = 18^\circ$ . Time for shaking 24 hours.

$C_{\text{phosphate}}$	$Z_{\text{N}}$	$Z_{\text{H}_3\text{PO}_4}$ ca.	$C_{\text{N}}$	$p_{\text{aH}}$	$Q_{\text{N}}$
—	46.2	2.0	8.4	3.796	0.182
49.2	—	2.4	19.0	3.698	0.411
49.8	—	2.8	29.6	3.608	0.641
—	92.4	2.0	2.6	4.040	0.028
49.2	—	4.0	53.3	3.648	0.577
49.8	—	4.8	73.3	3.551	0.793

As will be seen from the table above and Fig. 2, the solubility depends, as before, on the quantity of precipitate, and  $C_{\text{N}}$  is very nearly proportional with  $Z_{\text{N}}$ , so that the values of  $Q_{\text{N}} = C_{\text{N}}/Z_{\text{N}}$  lie in the same curve. The agreement between the determinations of phosphate ion concentrations is satisfactory.

It will also be seen from comparison of Fig. 1 with Fig. 2 that the solubility of the casein is the same in  $\text{HCl}$  and  $\text{NaCl}$  as in  $\text{H}_3\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$ . Further investigation in this direction is postponed until a later occasion.

## 5. Concluding Remarks.

After what we have seen in the foregoing, there can hardly be any doubt that casein, as liberated at isoelectric point, is a mixture of two or more components united in the precipitate by the

forces assumed by Sørensen<sup>1</sup>). On treating the precipitate with highly diluted hydrochloric acid it is very difficult to separate the components one from another, but something can be extracted after long subjection to the process, chiefly the more easily soluble portions (Precipitate Z). If stronger acid is used, the dissolution proceeds much more rapidly, and the casein may be completely dissolved. Something of the same kind is found on the alkaline side of the isoelectric point, but here, the surplus of base need only be very slight, and the casein seems also to behave differently as compared with the acid side, which may perhaps explain the results found by Cohn and Hendry (pag. 32).

On precipitating an acid solution of casein with a base, or a basic casein solution with a surplus of acid, the less soluble components or component groups are found mainly in the precipitate, the more soluble in the solution.

Neither the casein in the precipitate nor that in the solution is however, homogeneous, as for D and E we continually find the same mixture phenomenon, that the solubility depends on the quantity of precipitate. I have thus not had pure components in any of the mentioned preparations (probably not even in Z). That they are so difficult to separate is doubtless due to the fact that they associate one with another.

As the solubility equilibrium can be reproduced both from the acid side (hydrochloric acid solution  $p_H = \text{abt. } 2$ ) and from the basic (solution of casein in NaOH,  $p_H = 6.8$ ) and as D has a higher, E a lower solubility than A and B, and as the mean value of their content of formol-titratable nitrogen does not exceed that of unfractionated casein, and finally, as all the fractions and preparations of casein give distinct coagulation with rennet, the probability that the components are formed by irreversible decomposition is extremely slight, and we may therefore, as above, assume that the qualities found are those of the casein.

The chemical differences of the component groups may be characterised partly by their different behaviour in regard to colloidal precipitation with calcium salts, partly by the proportion between phosphorus and nitrogen, as previously utilised by Sørensen for characterisation of the globulins.

<sup>1</sup>) See p. 46.

The similarity between the behaviour of casein and the globulins thus supports the views of Sorensen with regard to the latter, and we cannot then but ask whether the proteins should generally be considered as mixtures. Further researches, which have already been commenced at this laboratory will, it is hoped, serve to decide the point.

*March 1925.*



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EN COMMISSION CHEZ H. HAGERUP

IMPRIMERIE DE THIELE

1925

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# STUDIES ON THE GROWTH OF SOME DANISH AGRICULTURAL PLANTS IN SOILS WITH DIFFERENT CONCENTRATION OF HYDROGEN IONS.

BY  
CARSTEN OLSEN.

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## Introduction.

During the past four or five years, American investigators in particular have made experiments with various cultivated plants in relation to the hydrogen ion concentration of the growth media. The experiments were made either with sand or water cultures, or by cultivation with soil as the medium.

Joffe (1920) has made experiments with alfalfa, cultivated in clay soil, the  $p_H$  value of which was varied by means of sulphuric acid and calcium carbonate within a range from 3.0 to 7.1. From a  $p_H$  value abt. 4, the alfalfa showed increasing growth with increase in the  $p_H$  value; the growth curve, however, shows a somewhat irregular course, and the hydrogen ion concentration of the soil had in some cases altered quite considerably during the progress of the experiment.

Sand culture experiments with maize, oats, wheat, alfalfa, red clover and soya beans have been carried out by O. C. Bryan (1922 and 1923). These experiments were made in tall, narrow glasses, each containing 600 cc of sand, which was watered with a nutrient solution in which the hydrogen ion concentration was varied, by addition of carbonate of soda or sulphuric acid, to give a difference of 1 in the  $p_H$  within a  $p_H$  range from 3—10. The nutrient solution was renewed each day by watering the sand all through with the solution, the surplus solution being drawn off from the bottom after passing through a filter there.

In the case of the oats, Bryan found that the strongest growth was obtained at  $p_H$  values between 6 and 7, the growth

decreasing gradually from  $p_H$  6—3.3 at which last value growth practically ceased. The wheat in Bryan's experiments behaved in much the same fashion, save that its growth decreased more markedly with increasing hydrogen ion concentration. Alfalfa was found to give strongest growth from  $p_H$  7—8, decreasing thence both with decreasing and increasing  $p_H$ . At  $p_H$  values of 5 and 6 Bryan obtained plants only half the bulk of those at  $p_H$  7 and 8.

O. Arrhenius has also (1922 and 1923) made experiments with alfalfa, as well as with sixteen other cultivated plants. He used for these experiments soil, the  $p_H$  value of which was varied by the addition of sodium hydroxide solution and sulphuric acid respectively. The results of his cultivation experiments with alfalfa will be seen from the figures below, showing the relative yield, in weight, of the plants at different  $p_H$  values of the soil

$p_H$	3—	4—	5—	6—	6.5—	7—	7.5—	8—	9
Relative Weight	12—	42—	56—	34—	40—	92—	91—	100—	92

If a growth curve be drawn on the basis of these figures, plotting the  $p_H$  values as abscissæ against the weights as ordinates, we find that the curve has two summits, one at  $p_H$  5 and another showing the optimal growth at  $p_H$  8. As not more than one experiment was made in each case, the degree of certainty is naturally not great, but Arrhenius nevertheless believes that the result is not due to experimental errors, but that the growth curve for alfalfa really has two summits. A great part of the other species used in his experiments also showed growth curves with two summits, one in the neighbourhood of  $p_H$  5, the other near  $p_H$  values 7 or 8<sup>1)</sup>. In the case of some species however, as for instance *Medicago lupulina*, he found a single-topped curve. It should here be noted that there seems to have been no investigation of the alterations of  $p_H$  which might possibly have taken place in the soil after the plants were sown, as the  $p_H$  value of the soil at conclusion of the experiment does not appear to have been measured. It must also be pointed out that substances like sulphuric acid solution and sodium hydroxide solution are extremely difficult to distribute evenly throughout the soil.

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<sup>1)</sup> Arrhenius supposes that one summit denotes the optimal concentration of hydrogen ions, the other the optimal concentration of hydroxyl ions for the plant in question.

Growth curves with two summits such as Arrhenius found for a great number of his experimental plants would hardly be expected.

As will be seen from the foregoing, alfalfa in particular has been subjected to cultivation experiments both in soil and in sand cultures of different hydrogen ion concentration, though the results cannot be said to show any high degree of conformity.

In addition to the growth experiments mentioned, there are in the literature statistical investigations, based on observations in the field, as to the growth of various agricultural plants in relation to concentration of hydrogen ions in the soil. These investigations, which will be dealt with in the following, were made principally with alfalfa and *Medicago lupulina*, and were carried out by K. A. Hasselbalch (1922), Maria Madsen (1922) and Harald R. Christensen (1924).

I have in a previous paper (Olsen, C. 1923) described water culture experiments made chiefly with wild plants, showing that some species (acid soil plants) attain their strongest growth in nutrient solutions whose  $p_H$  value lies about 4, whereas others (alkaline soil plants) attain their strongest growth in nutrient solutions with  $p_H$  between 6 and 7. According to the literature quoted above, most cultivated plants seem to behave as alkaline soil plants.

In the present paper, I shall describe a series of experiments carried out during the summers of 1922, 1923 and 1924, in order to investigate the growth of certain Danish agricultural plants in soils with different concentration of hydrogen ions. The idea was more especially to ascertain whether these plants differed to any essential degree in this respect. We could not naturally expect to find pronounced acid soil plants among our cultivated species<sup>1)</sup>, most of which must be presumed to thrive best in neutral soil; I should however, expect that the different species would vary considerably in their power of thriving on more acid or more alkaline soils. Alfalfa (*Medicago sativa*), *Medicago lupulina* and partly barley (*Hordeum distichum*) are generally designated as distinctly calciphilous plants, and we should therefore expect them to show a very slight power of thriving on acid soil. As alfalfa

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<sup>1)</sup> Highly acid soil plants are also cultivated in America; in some regions, for instance, unsuited for other cultivation, species of blueberry is grown; the *Vaccinium corymbosum*, which only thrives in highly acid soil (Coville, 1921).

plays a great part in our agriculture, there was particular reason for including this plant in our experiments, and it would also be interesting to ascertain how far this species really did show a two-topped curve as found by Arrhenius in a marked degree. The three above mentioned species were therefore included in all experiments, and as species which in contrast to these would be expected to thrive well in acid soil, I chose rye (*Secale cereale*) and buckwheat (*Fagopyrum sagittatum* var. *argenteum*).

## 1. Arrangement of the Experiments.

The experiments were made in vessels with soil as the medium. In experiments of this sort, it is important to treat the soil in a manner not too widely different from that of the agricultural land; it was therefore decided not to use sulphuric acid or sodium hydroxide solution for altering the concentration of hydrogen ions, these substances being also very difficult to distribute evenly throughout the medium. It is far better to start with a highly acid soil, and vary the hydrogen ion concentration by adding varying quantities of finely pulverised calcium carbonate. Sandy soil is preferable, as a pulverised material is far more easy to mix thoroughly in this than in a clay soil, in addition to which it will hardly be possible to obtain a clay soil of high acidity.

We therefore used for these experiments a highly acid sandy mould, rich in humus, the water extract of which showed a  $p_H$  value = 4.0. The soil was taken from a raspberry locality at Gadevang, in Grib forest, from the upper layer, 25 cm deep, and sent in sacks to the Carlsberg Laboratory; on arrival here, it was carefully sifted and mixed so as to render the whole as homogeneous as possible. Preliminary experiments with small quantities of the soil were made in order to ascertain how far the  $p_H$  of the water extract altered on adding different quantities of calcium carbonate.

The first step was to make a titration with  $n/25$  calcium hydroxide solution, taking samples of 50 g of soil and adding different quantities of calcium hydroxide solution, filling up with water thereafter to make the total quantity of liquid added equal 125 cc. After standing till next day, the mixture was filtered, and the  $p_H$  value of the extract colorimetrically determined (see Olsen C., 1923 p. 14). The  $p_H$  value



of the extract was then constant, as it did not alter on further standing. If a curve be then drawn, plotting  $p_H$  values as abscissæ against the added quantities of calcium hydroxide as ordinates we can of course read from this how much calcium hydroxide must be added to 50 g of soil to give any desired  $p_H$  value of the water extract. If, however, instead of calcium hydroxide, we add the equivalent amount of calcium carbonate to the soil (without adding water) and then, a week after, measure the  $p_H$  of the soil in water extract in the usual manner, we obtain a lower  $p_H$  than that given to the soil by the calcium hydroxide, and this  $p_H$  is not altered by leaving the soil to stand further. We have thus to add a greater quantity of calcium carbonate (abt. half as much again) than that which can be calculated by means of the curve, and the reason for this is probably that the carbonic acid from the calcium carbonate does not, as assumed in the calculation, entirely escape, but is partly bound by the soil.

It was therefore necessary for the preliminary experiments to apply the calcium carbonate directly. I then added to samples of 1 kg of soil different quantities of finely pulverised calcium carbonate, which were mixed very carefully with the soil. After standing for a week, the water extract of the soil gave a constant  $p_H$  value, which did not alter to any essential degree when the soil was left to stand further. A curve was then drawn, with the  $p_H$  scale as abscissa and the quantities of calcium carbonate as ordinates (Fig. 1) and by means of this curve we determined what amounts of calcium carbonate must be used for the final experiments with the plants.

In 1922, 1923 and 1924, stocks of soil were brought in from the mentioned locality in Grib forest, taken on precisely the same spot each year. It was necessary however, in the case of each batch, to make preliminary experiments with the addition of calcium carbonate, as the three lots were not so exactly identical as to demand exactly the same quantity of calcium carbonate to give the same alteration in the  $p_H$ . Fig. 1 for instance shows the titration curve for the soil taken in the spring of 1923. The figures on which this curve is based will be seen in Table 1. The  $p_H$  values in this table are arrived at as the mean of three measurements, the difference between them only in two cases exceeding 0.2 in the  $p_H$  and in the other cases being only 0.1 in  $p_H$ . In each of the three cases, after the batch of soil had been sifted and well mixed, I tried whether the addition of the same quantity of calcium carbonate to the same weight of soil, but taken from different parts of the stock, gave the same

Table 1.

g. Ca CO <sub>3</sub> per kg soil	0	1	2	3	4	5	6	10	50
pH.....	4.00	4.57	5.07	5.63	6.13	6.63	6.97	7.40	7.50

pH value; this was, practically speaking always found to be the case, the difference never exceeding 0.2 in pH, which seems to show that our mixture really was homogeneous throughout.

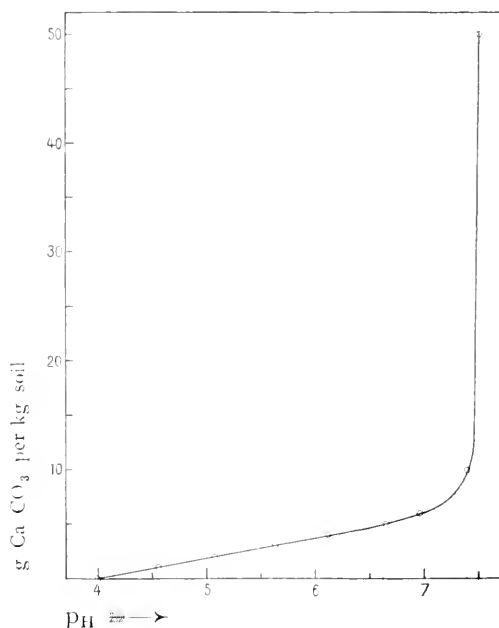


Fig. 1.

Titration curve for the soil used in experiments with alfalfa and rye.

As will be seen from the curve in Fig. 1, it was possible, by means of the calcium carbonate, to vary the pH of the soil in question within a range from 4.0—7.5. There was no occasion to cultivate plants in soil with pH value below 4, as such agricultural land is hardly found in Denmark. It would, on the other hand, be of interest to make cultivation experiments in soil with pH above 7.5; and this has also been done in one case, to wit, in the experiments with alfalfa, which was cultivated

in soil with a pH value of 8.5. I could not, however, with calcium carbonate, give the soil used a pH essentially higher than 7.5 and therefore used burnt lime. But as this in course of time takes up carbonic acid, which reduces the pH of the soil, I had, in these experiments, to water the soil thoroughly with diluted lime water, of a pH value abt. 8.5.

In the experiments with *Medicago lupulina*, I used earthenware jars glazed on the inside, holding each 4 kg of soil. In the experiments with the four other species I used ordinary flower

pots, likewise containing 4 kg of soil. A small piece of iron wire netting was placed over the hole in the bottom of the pot, and a piece of coarse canvas above this, and the pot then filled with soil. The flower pots were placed in saucers glazed on the inside, to retain any water that might run through. This water was then poured back on to the soil in the pot when it needed watering. Flower pots give better conditions for growth than the glazed earthenware pots, and even if they should absorb a little of the nutrient solution with which the plants were watered, this would not matter, as the plants were given an abundant surplus of nutritive matter during the experiments.

The soil was mixed with calcium carbonate in its natural moist state; drying the soil in such experiments must be regarded as quite unwarrantable. Particularly pure, finely pulverised precipitated calcium carbonate was used, and this was passed through a hair sieve to get rid of any lumps. In each case, the quantity of calcium carbonate to be added to the soil was then carefully mixed, first in a small portion of the soil; the rest of the soil was afterwards added, and the whole thoroughly mixed together. The quantity of soil to be used for each separate pot was treated by itself. The superficial area of the soil both in glazed jars and flower pots was abt. 225  $\square$  cm.

All the experiments were made in triplicate. The seeds were in all cases sown directly into the pots, and as soon as they had germinated, the plants were thinned out, so as to give an equal number of plants in each pot for each species. These were kept during the period of the experiment in the open air under a penthouse roof, formed of glass forcing frames, which could be removed when the weather was favourable. The plants were watered regularly throughout the entire period with distilled water.<sup>1)</sup>

In the case of *Medicago lupulina*, each jar was given, at the time of sowing, 0.5 g  $\text{KNO}_3$  and 0.5 g  $\text{KH}_2\text{PO}_4$  with the water used for watering. A month later, a further 0.5 g  $\text{KH}_2\text{PO}_4$  was added. In the case of the remaining species, I added, three times during the period of experiment, commencing just after sowing, a nutrient solution to the watering water, so as to give each pot at each watering 0.5 g  $\text{KNO}_3$ , 0.25 g  $\text{CaSO}_4$ , 2  $\text{H}_2\text{O}$ ,

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<sup>1)</sup> Watering with ordinary tap water was found to occasion a gradual rise in the pH of the soil, owing to the amount of lime contained.

0.25 g  $\text{KH}_2\text{PO}_4$ , 0.25 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 0.05 g  $\text{NaCl}$ . In the case of the alfalfa however, the sodium nitrate was omitted on the second and third occasions, primary potassium phosphate being added instead (0.5 g).

The pots were constantly changed about so as to alter their relative positions during the whole period of experiment, in order to counteract any possible difference in the conditions as to light and air.

At the close of the experiment, the plants were harvested, by cutting them off with a pair of scissors just at the surface of the soil. The plants were weighed, first in a fresh state, and later after having been dried in air (at  $20^\circ\text{C}$ ). The results will be seen from the following tables, showing the weight of dry matter for the parts above ground from each pot separately, as well as the average weight of dry matter of the plant mass from the three parallel experiments with soil of same  $p_{\text{H}}$  value. Further, I have noted for each species the relative dry weight obtained by taking the dry weight of the plants from the soil mixture in which the species attained its strongest growth as = 100, the remaining figures being reckoned in proportion. These figures are used in the following for the graphs for growth results. Further, the tables give the weight of the plants in a fresh state (mean of three parallel experiments) and the percentage of dry matter.

After the plants had been cut, the soil was sifted off from the roots and its  $p_{\text{H}}$  value measured colorimetrically for each pot. The figures noted in the tables as  $p_{\text{H}}$  value of the soil at conclusion of experiment represent the mean of the three figures<sup>1)</sup> obtained by measuring the soil from the three parallel experiments. On comparing the  $p_{\text{H}}$  values of the soil before commencing the experiment with those after conclusion of same (see following tables) it will be seen that alterations have taken place to some extent, but these are as a rule only slight. The greatest difference amounts to 0.5 in  $p_{\text{H}}$ . The alteration in most cases amounted to a rise of the  $p_{\text{H}}$  value during the period of experiment, possibly owing to the fact that the soil was manured with nitrogen in the form of potassium nitrate. The tables show the mean of the  $p_{\text{H}}$  values prior to commencement of the experiment and

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<sup>1)</sup> These figures differed but very slightly. Greatest difference was 0.3 in  $p_{\text{H}}$ .

after conclusion of same. These figures are taken as the average  $p_H$  values of the soil during the period of cultivation, and therefore employed in the graphs hereafter given.

We may now proceed to consider the results of the experiments with the separate species.

## 2. Experiments with Alfalfa (*Medicago sativa*).

The seeds were sown on the  $15/5$  1923, after previous inoculation with a bacteria culture from root tubercles of alfalfa<sup>1)</sup>. The plants were cut on the  $29/9$  1923. After the roots had been taken up, it was found that root-tubercles had formed throughout; in the most acid soil, however, the root-tubercles were few in number and but slightly developed. For the rest, the seeds germinated equally well in all cases, and the appearance of the seedlings was uniform in essentials; only in the most acid soil were they smaller and of a darker green than in the other pots. Even in the most alkaline soil, no chlorosis was observed.

The experimental results are shown in Table 2, and a graph in Fig. 2. The growth curve here given shows a very regular course, with summit at about  $p_H$  6.5—7.1. From here it falls away to either side, but most markedly with decreasing  $p_H$  value. At a  $p_H$  value of 5.0, the weight of the plant mass has already fallen to less than half, and at  $p_H$  4.0 it is only abt. 13 % of the weight at optimal concentration of hydrogen ions. This agrees well with practical experience, as the plant is known to thrive but poorly where the soil is lacking in lime. As will be seen from the figure, however, the weight of the plant mass decreases quite appreciably with increasing alkaline reaction of the soil. The species has thus a somewhat restricted range of  $p_H$  in which the growth is optimal or approximately so. As will be seen, there is nothing to indicate any double summit to the growth curve, as suggested by Arrhenius.

In regard to alfalfa, we have some statistical investigations as to the growth of this plant in cultivated soils with different hydrogen ion concentration, based on examination of alfalfa fields in this country, where the  $p_H$  value was measured and an esti-

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<sup>1)</sup> The bacteria cultures for alfalfa and *Medicago lupulina* were kindly furnished by Frk. cand. pharm. Maria Madsen, of the Agricultural Laboratory.

**Table 2.**

Cultivation of alfalfa in soils with different concentration of hydrogen ions.

pH of soil	before exper.	4.0	5.0	6.0	6.5	7.0	7.5	8.5
	after —	4.4	5.4	6.5	6.8	7.2	7.8	8.2
	mean .....	<b>4.20</b>	<b>5.20</b>	<b>6.25</b>	<b>6.65</b>	<b>7.10</b>	<b>7.65</b>	<b>8.35</b>
Weight of plants in a fresh state. Mean, g .....		5.7	12.0	19.3	22.3	20.3	19.3	13.8
Dry weight of plants in g	pot no. 1....	2.00	3.95	5.47	6.80	6.38	6.50	4.65
	— 2....	1.35	3.00	6.50	6.71	6.65	5.62	4.87
	— 3....	1.50	3.50	5.95	6.70	6.42	6.00	3.90
	mean .....	1.62	3.48	5.97	6.74	6.42	6.04	4.47
Relative weight of dry matter.		<b>24.0</b>	<b>51.6</b>	<b>88.6</b>	<b>100.0</b>	<b>95.2</b>	<b>89.6</b>	<b>66.3</b>
Percentage of dry matter in the plants.....		28.4	29.0	30.9	30.2	31.6	31.3	32.4
P <sub>2</sub> O <sub>5</sub> percentage of dry matter		0.51	0.58	0.57	0.56	0.58	0.53	0.41
Ca — — —		1.6	1.7	1.6	1.7	1.7	2.0	2.2
N — — —		3.0	3.2	3.2	3.2	3.2	3.3	3.2

mate formed at the same time as to the condition of the plants there grown. According to these investigations, the work of K. A. Hasselbalch (1922 and Maria Madsen (1922) alfalfa on the whole thrives best in soil with a p<sub>H</sub> value between 7.0 and 7.4.

After the plants had been cut, and the parts dried and weighed, the quantity of nitrogen, phosphoric acid and lime in the dried material was determined.

A suitable weighed quantity of the plant mass was decomposed by boiling with concentrated sulphuric acid (digestion) in which a small piece of copper wire was placed. After a fairly long period of boiling, a transparent pale blue liquid was obtained. Part of this was used for nitrogen determination by the Kjeldahl method, another for alkali-metric measurement of phosphoric acid after the Neumann and Gregersen method (Gregersen 1907) and finally, a third portion was used for calcium determination. The calcium was precipitated as oxalate, and this again after thorough washing, titrated with potassium permanganate solution. Double determinations were made throughout.

The result will be seen from Table 2, which shows that while the nitrogen percentage of the alfalfa does not vary with the concentration of hydrogen ions in the soil, the calcium content of this plant increases when grown in soil of any p<sub>H</sub> essen-

tially exceeding 7.0, the amount of phosphoric acid, on the other hand, decreasing, though not in any essential degree until the soil has reached a  $p_H$  value of abt. 8.0 or more. That the plant finds difficulty in absorbing phosphoric acid from the soil when

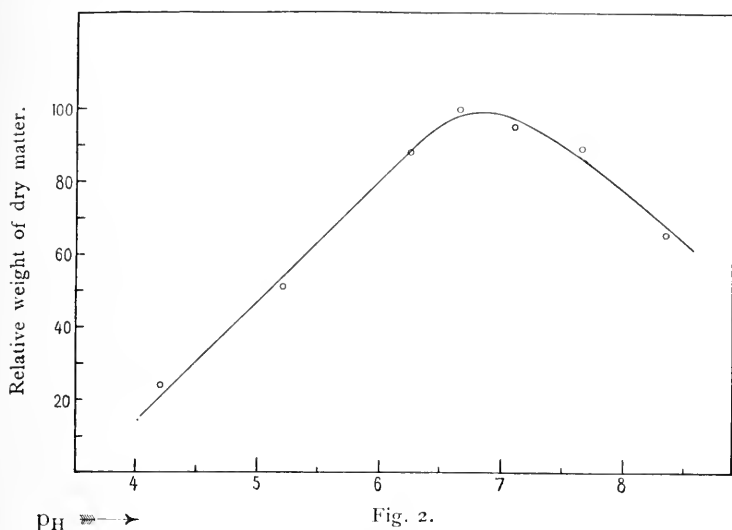


Fig. 2.

Alfalfa (*Medicago sativa*). Growth in soils with different concentration of hydrogen ions.

the  $p_H$  value of the latter is much above 7.0 is not difficult to understand, as the phosphoric acid would in such case be precipitated out as normal calcium phosphate, which is practically insoluble in water. That the calcium content of alfalfa should rise when the soil in which it grows has a  $p_H$  value much above 7.0, is hardly connected with the greater quantity of calcium in the soil, as only a very slight amount of this of course is in solution. In Bryan's sand culture experiments, the amount of calcium was the same throughout, and he found that red clover, soya beans and alfalfa took up the more calcium the higher the  $p_H$  of the nutrient liquid. With the cereals, on the other hand, this was not the case in Bryan's experiments.

### 3. Experiments with *Medicago lupulina*.

The seeds were sown on the 17/5 1922, having been inoculated with a bacteria culture from root tubercles of *Medicago lupulina* immediately beforehand. The experimental results will

Table 3.

Cultivation of *Medicago lupulina*, in soils with different concentration of hydrogen ions.

pH of soil	before experiment ...	4.02	5.04	6.94	7.50
	after — ...	3.93	5.10	6.82	7.43
	mean .....	<b>3.97</b>	<b>5.07</b>	<b>6.88</b>	<b>7.46</b>
Weight of plants in fresh state. Mean,					
g .....		26	106	179	153
Dry weight of plants in g	Jar no. 1 .....	5.0	26.0	47.0	37.0
	— - 2 .....	6.0	23.0	39.0	36.0
	— - 3 .....	5.7	29.0	39.0	38.0
	mean .....	<b>5.57</b>	<b>26.0</b>	<b>41.7</b>	<b>37.0</b>
Relative weight of dry matter.....		<b>13.4</b>	<b>62.4</b>	<b>100.0</b>	<b>88.7</b>
Percentage of dry matter in the plants.		21.4	24.5	23.3	24.2

be seen from Table 3, and a graph is shown in Fig. 3. Owing to an error, the plants were not cultivated in soil of pH value 6.0, and the curve for the pH area from 5.5—6.5 is therefore drawn as a dotted line, as there should have been a point here. It is hardly possible however, that this should have occasioned any essential alteration in the course of the curve. With regard to root tubercles, the plants behaved exactly as alfalfa (above noted). Germination was good and uniform throughout, and, as in the alfalfa experiments, the seedlings in the most acid soil appeared smaller and darker in colour than those in the other pots. The plants flowered in all except the most acid soils, and seeds ripened before they were cut.

The growth curve shows a very regular course, with summit between pH 6.5—7.0. It shows remarkable similarity to the curve for alfalfa — the two plants are, of course, very closely related.

Harald R. Christensen (1924) has made researches with regard to the behaviour of *Medicago lupulina* in cultivated soil of different hydrogen ion concentration, based on investigation of a great number of fields in this country, measuring the pH value of the soil and at the same time estimating the growth of the plants. Soils with pH at or about the neutral point (pH = 6.6—7.3) gave the greatest number of cases with good development, and soils of lower and higher pH values showed less fa-



vourable results. The result of these researches thus agrees well with the results obtained by the above mentioned cultivation experiments.

Similar investigations to those made by Christensen have

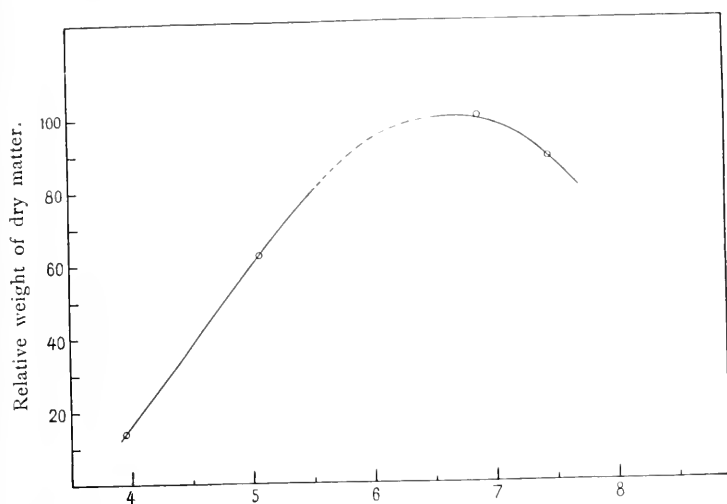


Fig. 3.

PH  $\Rightarrow$  *Medicago lupulina*. Growth in soils with different concentration of hydrogen ions.

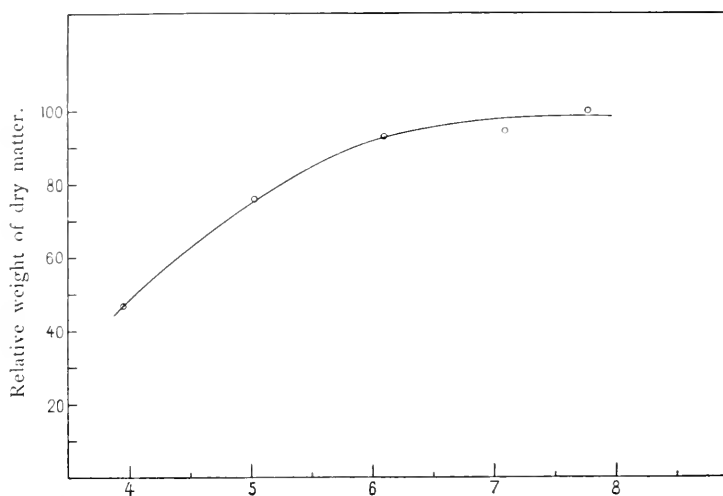
also been carried out by K. A. Hasselbalch (1922) and Maria Madsen (1922). Both these writers found that *Medicago lupulina* thrived best in cultivated soils of pH between 7.0 and 7.4.

#### 4. Experiments with Barley (*Hordeum distichum*).

The strain used for these experiments was Tystofte Prentice. The seeds were sown on the 12/5 1924, and the plants cut on the 2/7 1924. Germination proceeded uniformly throughout, but after only a week the growth was already distinctly different according to the concentration of hydrogen ions in the soil.

The experimental results are shown in Table 4 and a graph in Fig. 4. The strongest growth lies in the pH area 6.5—8.0; at higher values than 8.0 the growth will probably decrease gradually, but nothing can be said from the experiment as to this, no soil with pH above 7.8 having been used here. The curve shows, however, that the growth from a pH value abt. 6.5 decreases

quite considerably with decreasing  $p_H$ , though not nearly so much as in the case of alfalfa and *Medicago lupulina*. The



$p_H \rightarrow$

Fig. 4.

Barley (*Hordeum distichum*). Growth in soils with different concentration of hydrogen ions.

barley is thus less sensitive to variation in the hydrogen ion concentration of the soil than the two leguminosa above mentioned.

Table 4.

Cultivation of barley in soils with different concentration of hydrogen ions.

		3.80	5.00	6.00	7.00	7.75
$p_H$ of the soil	before experiment....	3.80	5.00	6.00	7.00	7.75
	after — ....	4.10	5.10	6.20	7.20	7.80
	mean.....	<b>3.95</b>	<b>5.05</b>	<b>6.10</b>	<b>7.10</b>	<b>7.78</b>
Weight of plants in fresh state. Mean,						
g.....		31.7	51.7	59.0	66.3	66.0
Dry weight of plants in g	Pot no. 1.....	3.20	6.10	7.00	8.35	7.20
	— - 2.....	4.00	5.50	7.30	7.20	8.80
	— - 3.....	4.05	6.60	8.05	7.00	8.00
	mean.....	3.75	6.07	7.45	7.52	8.00
Relative weight of dry matter .....		<b>46.9</b>	<b>75.9</b>	<b>93.1</b>	<b>94.0</b>	<b>100.0</b>
Percentage of dry matter in the plants.		11.8	11.7	12.6	11.3	12.1

### 5. Experiments with Rye (*Secale cereale*).

Seeds were sown on the  $\frac{8}{5}$  1923, the plants were cut on  $\frac{8}{8}$  1923, and all plants had by that time ripe fruits. After only ten days from sowing, plants in soil with  $p_H$  6.25 showed the strongest growth.

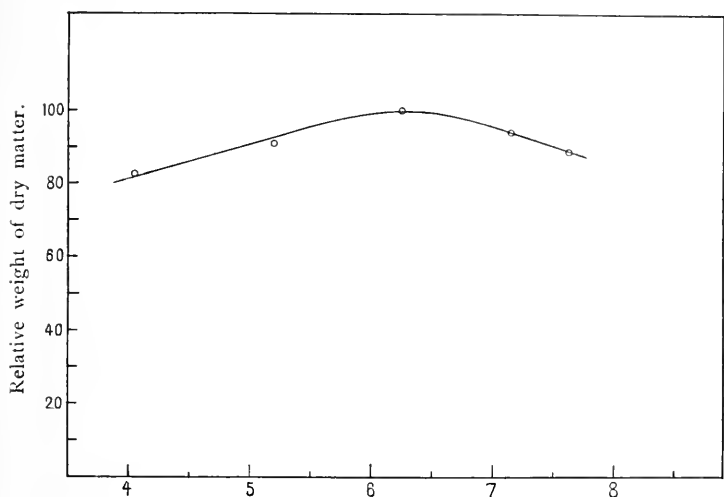


Fig. 5.

Rye (*Secale cereale*). Growth in soils with different concentration of hydrogen ions.

The experimental results are shown in Table 5, and a graph is given in Fig. 5. The summit of the growth curve lies at a  $p_H$  value of abt. 6.2, the growth decreasing thence to either side.

Table 5.

Cultivation of rye in soils with different concentration of hydrogen ions.

pH of soil		4.00	5.00	6.00	7.00	7.50
{	before experiment....	4.00	5.00	6.00	7.00	7.50
	after — ....	4.13	5.43	6.50	7.33	7.77
	mean.....	4.06	5.22	6.25	7.16	7.63
Weight of plants in fresh state. Mean g.....		56.3	62.3	73.7	67.0	65.0
Dry weight of plants in g	Pot no. 1.....	14.1	16.0	18.7	17.0	15.6
	— - 2.....	15.4	16.4	16.7	15.2	17.1
	— - 3.....	14.7	16.2	18.1	16.0	15.1
	mean.....	14.7	16.2	17.8	16.1	15.9
Relative weight of dry matter.....		82.6	91.0	100.0	90.4	89.3
Percentage of dry matter in the plants.		26.1	26.0	24.2	24.0	24.5

Rye, however, appears far less sensitive to variation in the hydrogen ion concentration of the soil than any of the three other plants above described. In soil with a  $p_H$  value of 4.0, for instance, the plant attained a weight amounting to 82 % of the weight of that from the soil in which strongest growth was found.

## 6. Experiments with Buckwheat (*Fagopyrum sagittatum* var. *argenteum*).

Seeds were sown in the  $14/5$  1924, and the plants cut on the  $3/7$  1924. The plants had then just begun to flower.

Table 6.

Cultivation of buckwheat in soils with different concentration of hydrogen ions.

pH of soil	{ before experiment....	3.80	5.00	6.00	7.00	7.75
	{ after — ....	4.10	5.00	6.30	7.15	7.83
	{ mean.....	<b>3.95</b>	<b>5.00</b>	<b>6.15</b>	<b>7.08</b>	<b>7.79</b>
Weight of plants in fresh state. Mean						
g.....		81.0	91.0	88.0	91.3	75.3
Weight of dry matter in plants in g	{ Pot no. 1.....	4.95	4.85	4.85	5.35	4.30
	{ — - 2.....	4.80	5.35	6.00	5.95	4.35
	{ — - 3.....	4.45	5.15	4.95	4.65	4.90
	{ mean.....	4.73	5.12	5.27	5.32	4.52
Relative weight of dry matter.....		<b>88.9</b>	<b>96.2</b>	<b>99.1</b>	<b>100.0</b>	<b>85.0</b>
Percentage of dry matter in plants...		5.8	5.6	6.0	5.8	6.0

The experimental results are shown in Table 6 and a graph is given in Fig. 6. The summit of the growth curve abt.  $p_H$  6—7. With rising  $p_H$ , the growth decreases considerably; with decreasing  $p_H$ , growth also decreases, but only to an insignificant degree compared with the species previously mentioned. In soil with  $p_H$  4.0 for instance, the growth is only 10 % below that of soil with  $p_H$  between 6 and 7. Buckwheat is thus but very slightly affected by a high concentration of hydrogen ions in the soil, while on the other hand it does not seem to favour soil with alkaline reaction. This is possibly connected with the high hydrogen ion concentration of the cell sap. The  $p_H$  value of the cell sap lies

abt. 4.4 as regards the stem and leaves. On testing with lakmoid paper, however (see Hempel 1916 p. 119) no difference could be discerned in the  $p_H$  value of the cell sap in the parts above ground between plants in soils of different hydrogen ion con-

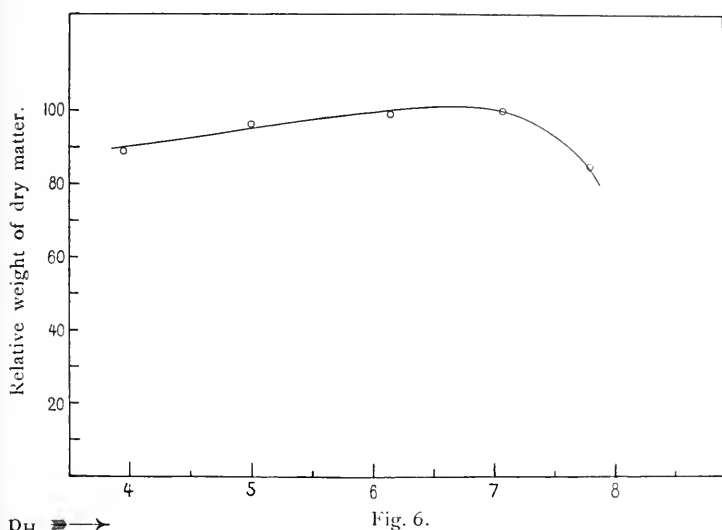


Fig. 6.  
Buckwheat (*Fagopyrum sagittatum* var. *argenteum*). Growth in soil with different concentration of hydrogen ions.

centration. The buckwheat experiments show that in a given species of plant, the normal hydrogen ion concentration of the cell sap and the hydrogen ion concentration of optimal soil may differ greatly.

## 7. Concluding Remarks on the Result of the Experiments.

As will be seen from the experiments above described, the five different cultivated plants here used show considerable difference in regard to the effect of variation in the hydrogen ion concentration of soil. The optimal range of concentration for the separate species shows considerable difference as between one and another. Alfalfa (*Medicago sativa*), for instance and *Medicago lupulina*, thrive best in soil with a  $p_H$  value of 6.5–7.0, barley (*Hordeum distichum*) best in soil with  $p_H$  6.5–8.0, rye (*Secale cereale*) best in soil with  $p_H$  value 6.0–6.5, and buckwheat (*Fagopyrum sagittatum* var. *argenteum*) best in soil with

$p_H$  value 6.0—7.0. Rye and buckwheat are far better able to stand higher concentration of hydrogen ions in the soil than the other three species; buckwheat and rye for instance, in soils of  $p_H$  4.0 attain a growth amounting to 90 and 82 per cent respectively of the growth at optimal concentration of hydrogen ions, whereas alfalfa and *Medicago lupulina*. at the same  $p_H$  value of soil, only show 13 and 16 per cent respectively of the growth at optimal concentration. There is thus considerable difference in the power of the different species to thrive in highly acid soils.

The different species' power of standing higher alkaline reaction of the soil, on the other hand, shows less difference, only the barley being something of an exception, as its growth does not seem to decrease in the range from  $p_H$  7.0—8.0. With the other species however, the growth decreases with increasing  $p_H$  value from abt.  $p_H$  7.0 upwards, so that the growth at  $p_H$  7.8 is abt. 80—85 % of that at optimal concentration of hydrogen ions. Even such decidedly calciphilous plants as alfalfa and *Medicago lupulina* were found to be sensitive to increasing alkaline reaction of the soil.

None of the cultivated plants here investigated showed any two-topped curve such as Arrhenius found in the case of several species, e. g. alfalfa.

The growth curves given above for the different species may be taken as generally valid, as it is hardly likely that the chemical and physical conditions of the soil would affect the course of the growth curves. It probably does not matter, for instance, whether sandy or clay soil be used. On the other hand, the growth curves can only be valid when the plants are given abundant plant food, as was the case in our experiments. Otherwise, the accessibility of the plant foods contained in the soil will differ at the same time, and we should then have not only direct, but also indirect effects of alteration in the hydrogen ion concentration. It is especially the accessibility of nitrogen which will be of importance. In the soil used for the experiments, which was rich in humus and highly acid, Nature has hardly provided any great quantity of nitrogen in the form of ammonia or nitrates, but on the other hand there will doubtless be a quantity of organically bound nitrogen, which, by the addition of calcium and consequent raising of the  $p_H$  value of the soil, is more rapidly transformed into inorganic nitrogenous compounds,

as the activity of the nitrogen-transforming organisms increases greatly with increasing  $p_H$  value of the soil. If the plants in the experiments had not been manured with nitrogenous matter, they would therefore probably, in soil of higher  $p_H$  value, have obtained more nitrogen in the form of inorganic compounds than in soil of lower  $p_H$  value. The growth curves would then presumably in such case fall even more sharply with decreasing  $p_H$ , than is the case with the curves given above. Carried out as these experiments were, with abundance of plant food, the decreasing growth with decreasing  $p_H$  of the soil must be directly due to the increase in hydrogen ion concentration as such, all secondary effects such as difference in quantity and accessibility of the plant food substances being here eliminated. We cannot however, say the same with regard to the decrease in growth with increasing concentration of hydroxyl ions. Whether we should here speak of a decrease in concentration or hydrogen ions, or an increase in the concentration of hydroxyl ions is a question which must be left open for the present, but the accessibility of the plant food substances can here also be of essential importance, as the solubility of iron and phosphorus decreases very greatly with increasing alkaline reaction of the soil. The iron is precipitated out as hydroxide or phosphate, and the phosphoric acid as normal calcium or ferri-phosphate. The experiments with alfalfa suggest that the phosphoric acid was hardly accessible to this plant in the alkaline soil mixtures, as plants from these contained less phosphoric acid than those from the remaining soils. No iron analyses were made, but as no chlorosis was observed in any of the five plants during the experiments, it does not look as if lack of iron had played any important part.

It will be evident from the experiments here made that in practical agriculture, even in the cultivation of such calciphilous plants as alfalfa and *Medicago lupulina*, it is possible to give the ground too much lime. This has perhaps hardly been sufficiently appreciated up to now. In the case of Danish soils, land with  $p_H$  value abt. 8.0 and over may occur, though not frequently, and so strong an alkaline reaction as this is hardly very favourable to any cultivated plant. It was formerly believed that neutral or slightly alkaline reaction to litmus solution was generally the most favourable for agricultural soil. As litmus solution

has its point of change at a  $p_H$  value of 6.5, a neutral — slightly alkaline reaction in litmus solution answers roughly to a  $p_H$  range from 6.5—7.0. This range will also be particularly favourable for the growth of the plants here dealt with; only in the case of rye would a somewhat lower  $p_H$  value of the soil be preferable. It would be of considerable interest however, to have further experiments made with a larger number of our agricultural plants than was possible in the present investigations.

*March 1925.*



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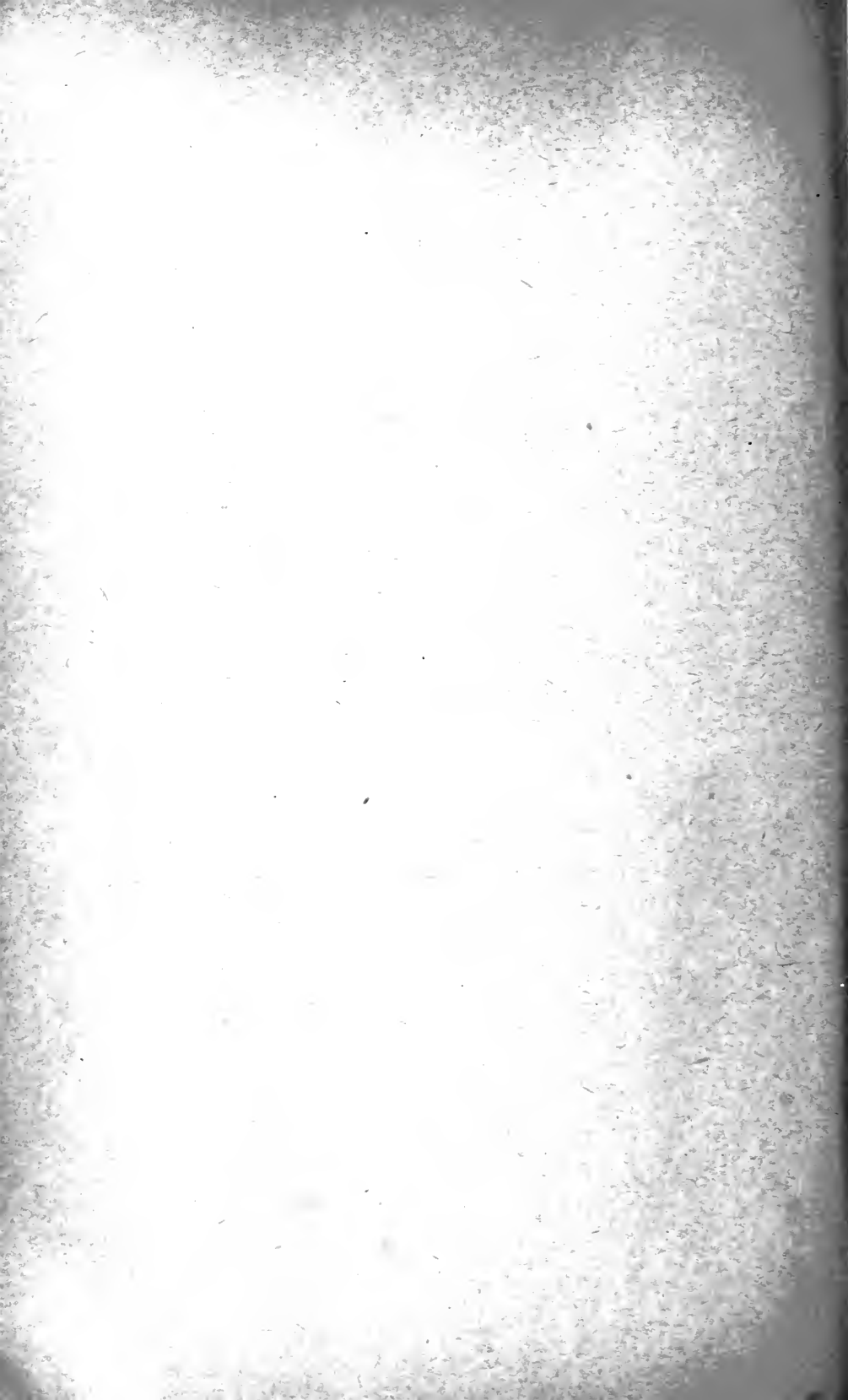
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# MEASUREMENTS WITH THE QUINHYDRONE ELECTRODE.

BY

K. LINDERSTRØM-LANG.

During the past year, we have been making experiments at the Carlsberg Laboratory with the Biilmann quinhydrone electrode<sup>1-10</sup>) with a view to its use in determining the concentration of hydrogen ions in protein solutions containing ammonium sulphate. It was soon found to fulfill all requirements in point of rapidity, and to give values which could be reproduced with a high degree of accuracy (abt. 0.01 pH). It was necessary, however, to investigate the inevitable errors<sup>2)</sup> <sup>6)</sup> involved in measuring solutions of even quite moderate concentration with the quinhydrone electrode. At the suggestion of Prof. S. P. L. Sørensen, therefore, the writer carried out the experiments described in the present paper. These include: new determinations of the difference of potential between the quinhydrone and the hydrogen electrode in hydrochloric acid-potassium chloride and sodium chloride solutions, as also determinations of the ammonium sulphate error and the protein error (in solutions of egg albumin, serum albumin and casein; as regards the last-named, see<sup>10)</sup>).

<sup>1)</sup> E. Biilmann: *Ann. de Chimie* **15**, 109 (1921).

<sup>2)</sup> S. P. L. Sørensen, M. Sørensen and K. Linderstrøm Lang: *Compt.-rendus du Lab. Carlsberg* **14**, No 14 (1921).

<sup>3)</sup> E. Biilmann et Hakon Lund: *Ann. de Chim.* **16**, 321 (1921).

<sup>4)</sup> S. Veibel: *Journ. Chem. Soc.* **123**, 2203 (1923).

<sup>5)</sup> E. Biilmann: *Trans. Faraday Soc.* **19**, Part 3, (1924).

<sup>6)</sup> K. Linderstrøm-Lang: *Compt.-rendus du Lab. Carlsberg* **15**, No 4 (1924).

<sup>7)</sup> E. Biilmann and Inger Krarup: *Journ. Chem. Soc.* **125**, 1954 (1924).

<sup>8)</sup> J. M. Kolthoff: *Zeitschr. f. physiol. Chemie* **144**, 259 (1925).

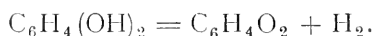
<sup>9)</sup> E. Schreiner: *Zeitschr. f. physikal. Chem.* **117**, 57 (1925).

<sup>10)</sup> K. Linderstrøm-Lang and S. Kodama: *Compt.-rendus du Lab. Carlsb.* **16**, No 1 (1925).

Such determinations enable us, with suitable corrections, to use the quinhydrone electrode for measurements which occur in protein investigations, and it must be added that the agreement between parallel determinations of hydrogen ion concentration obtained by this means is superior to that given by the ordinary hydrogen electrode (see K. Linderstrøm-Lang and S. Kodama l. c.). The quinhydrone electrode may, then — if used with care and previously tested — be advantageously used in place of the hydrogen electrode, though the latter must still be regarded as the standard electrode.

### A. Difference between the Potential of the Quinhydrone Electrode and the Hydrogen Electrode in Dilute Electrolyte Solutions.

The fundamental reaction in the quinhydrone electrode is as we know:



If we now designate the activity, the activity coefficient, and the concentration of hydroquinone and quinone  $a_{\text{H}}$ ,  $f_{\text{H}}$  and  $c_{\text{H}}$ ,  $a_{\text{K}}$ ,  $f_{\text{K}}$  and  $c_{\text{K}}$  respectively, then the following equations will apply exactly:

$$a_{\text{H}_2} = k \cdot \frac{a_{\text{H}}}{a_{\text{K}}} \quad p_{\text{H}_2} = k' \cdot \frac{c_{\text{H}} \cdot f_{\text{H}}}{c_{\text{K}} \cdot f_{\text{K}}} = k' \cdot \frac{f_{\text{H}}}{f_{\text{K}}},$$

$p_{\text{H}_2}$  being the hypothetical hydrogen pressure of the quinhydrone electrode, and  $c_{\text{H}} = c_{\text{K}}$ . The potential  $E_{\text{KB}}$  of the following element: Pt, quinhydrone, experimental liquid,  $\text{H}_2$ , Pt (1 atmosphere) 18° (1) is therefore

$$E_{\text{KB}} = -0.02885 \cdot \log \frac{p_{\text{H}_2}}{1} = -0.02885 \cdot \log k' - 0.02885 \log \frac{f_{\text{H}}}{f_{\text{K}}}, \quad (1)$$

where  $f_{\text{H}}$  and  $f_{\text{K}}$  are the activity coefficients in the experimental liquid.

As the concentrations of hydroquinone and quinone in the well known experimental method employed are very slight,  $f_{\text{H}}$  and  $f_{\text{K}}$  in pure water as experimental liquid will both be 1, and  $E_{\text{KB}}^0$  is therefore determined by:

$$E_{\text{KB}}^0 = -0.02885 \cdot \log k' \quad (2)$$

For practical reasons, however, it is impossible to use pure water as experimental liquid, and we have therefore been obliged



to use a dilute electrolyte solution of well defined hydrogen ion concentration, e. g. 0.01 n HCl, 0.1 n HCl, 0.01 n HCl + 0.09 n KCl, Sørensen's phosphate mixtures etc.  $E_{KB}$ , the value of element (I) with the first-mentioned experimental liquid 0.01 n HCl, has previously been determined (Sørensen, Sørensen and Linderstrøm-Lang l. c.) as 0.7048 volts, whereas Biilmann and Lund give for the same solution, originally 0.7044, Biilmann (l. c.), Veibel (l. c.) and Biilmann and Krarup (l. c.) give for 0.1 n HCl 0.7044, for 0.01 n HCl + 0.09 KCl, 0.7040 and 0.7042, and for phosphate mixture ( $p_H = 6.81$ ) 0.7045. The writer has himself, with S. Kodama, determined the value of  $E_{KB}$ , using 0.01 n HCl + 0.09 n KCl for experimental liquid, as 0.7038, and suggested the possibility that this low value might be due to evaporation of the quinone in the course of the drying process following on the production of the quinhydrone (see Biilmann and Lund l. c.). This, as will be seen from the following, is hardly correct.

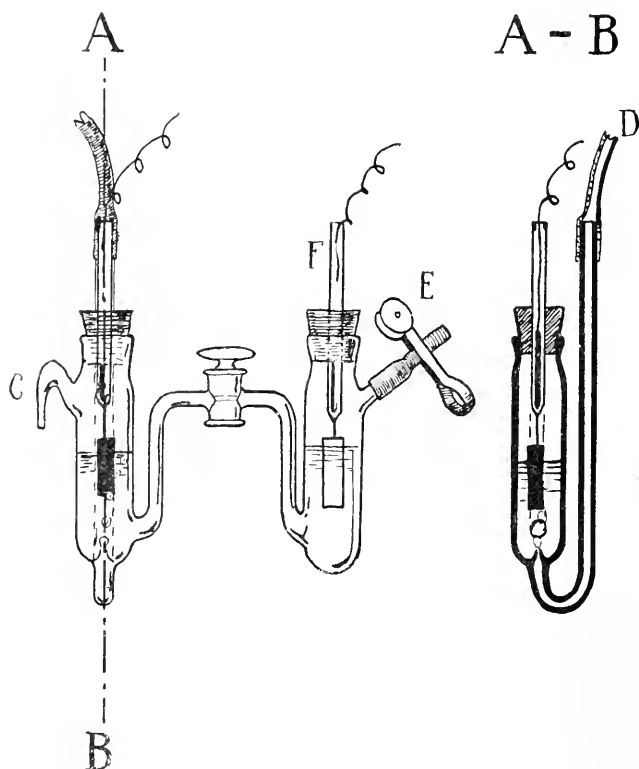
The differences between the values thus found are of no essential importance in practical work with the quinhydrone electrode, except in cases where a particularly high degree of accuracy is required. It is, however, desirable that the starting point of this important experimental method should be as clear as possible, and I have therefore thought it best to subject the value found at this laboratory for 0.01 n HCl, viz. 0.7048, to a further test, using a somewhat different experimental method; and at the same time, by measuring  $E_{KB}$  with liquids of varying electrolyte content, endeavoured to throw light on the divergencies found. For, as will be seen on comparing equations (1) and (2), we can only expect to get the same value for  $E_{KB}$  with the different experimental liquids above noted, if we can assume that  $f_H = f_K$  within the limit of accuracy involved in method of the measurement. If this be not the case; if  $f_H$  and  $f_K$  are different, and measureably different, functions of the electrolyte content, then we have here a possible explanation of the divergence. As will be seen from the following, the influence of the electrolytes is really the principal source of difference.

#### *Method of measurement.*

Fig. 1 shows the apparatus employed. It consists simply of a hydrogen electrode connected with a quinhydrone electrode. The apparatus is placed in a water thermostat at 18°, immersed

to the point at which the water level in the thermostat coincides with that of the liquid in the vessels. A short rubber tube is attached to the tube C, dipping into the water for abt.  $\frac{1}{2}$  cm and acting as a water lock. The hydrogen enters at D and passes out through C.

Fig. 1.



(Half size).

The vessel is first thoroughly washed three or four times with the experimental liquid, and the electrodes are left to stand in this for about 10 minutes before being fixed in their places; the tap is turned off, and the whole apparatus placed in the thermostat. After the hydrogen has bubbled for abt. half an hour, the clip E is taken off, the electrode F removed, and a little quinhydrone added and stirred up in the liquid with the electrode, which is then placed in it. The clip E is then replaced, the tap turned on, and the apparatus is ready for measurement.

The hydrogen was produced by electrolysis, and twice was-

hed in potassium pyrogallate, and subsequently in water, before being introduced into the vessel.

The quinhydrone was prepared by the Biilmann and Lund method, and the preparation marked C. L. VII was old, and identical with that used by Linderstrom-Lang and Kodama, freshly prepared by them at that time.

C. L. VIII was freshly prepared, likewise according to the Biilmann-Lund method, but was also recrystallised by alcohol and dried for 45 minutes between filter papers.

Three quinhydrone electrodes and three hydrogen electrodes were used, the same throughout all the series of experiments.

The potential measurement was effected by means of an Otto Wolff potentiometer, with a mirror galvanometer as zero instrument.

The three following experiments are quoted as showing the constancy of the elements. The time is that which elapsed after adding the quinhydrone.

0.01 n HCl + 0.09 n KCl		0.05 n HCl		0.1 n HCl	
Time Min.	E	Time Min.	E	Time Min.	E
3	(0.70370)	1	0.70409	1	0.70400
8	0.70385	9	0.70408	2	0.70396
14	0.70381	18	0.70408	10	0.70394
26	0.70390	26	0.70404	17	0.70402
36	0.70386	37	0.70411	21	0.70402
45	0.70387	48	0.70414	32	0.70406
54	0.70385	60	0.70414	41	0.70404
62	0.70389	71	0.70416	49	0.70403
68	0.70385	80	0.70415	59	0.70403
mean 0.70386		mean 0.70411		mean 0.70401	

Corrected for barometric pressure and water vapour content:

0.70424

0.70453

0.70443

### *Results of the Measurements.*

The results of the experiments, comprising the experimental liquids: 0.01 n HCl, 0.02 n HCl, 0.05 n HCl, 0.10 n HCl and the mixture 0.01 n + 0.09 n KCl, will be found in Table I.

Table 1.  
Quinhydrone electrode — Hydrogen electrode.

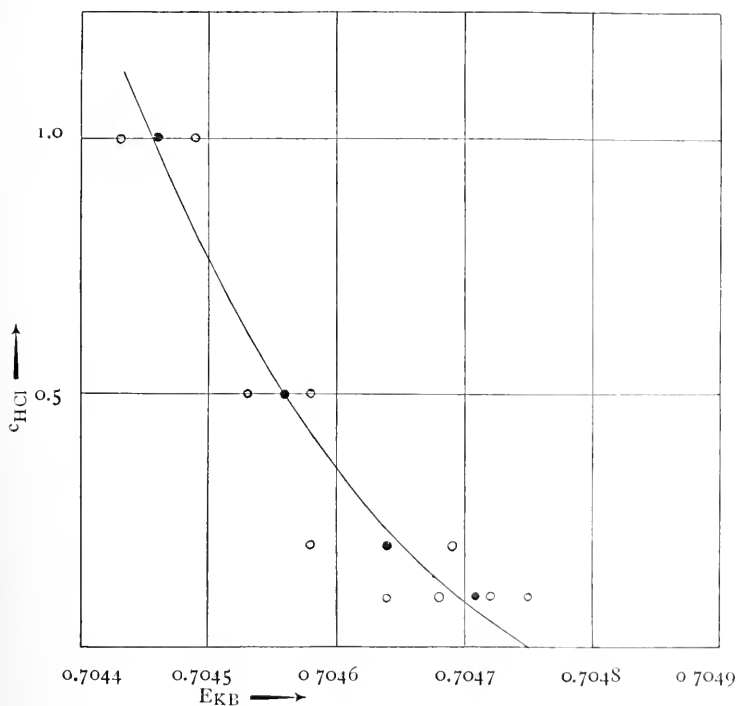
Experimental liquid	Quinhydrone	EKB 18°, dry hydrogen	EKB mean
A.			
0.01 n HCl	C. L. VIII	0.70472	0.70471
		0.70475	
		0.70468	
		0.70475	
		0.70464	
0.02 n HCl	C. L. VIII	0.70469	0.70464
		0.70458	
0.05 n HCl	C. L. VIII	0.70458	0.70456
		0.70453	
0.10 n HCl	C. L. VIII	0.70443	0.70446
		0.70449	
0.01 n HCl + 0.09 n KCl	C. L. VIII	0.70420	0.70423
		0.70411	
		0.70427	
		0.70432	
		0.70424	
B.			
0.01 n HCl + 0.09 n KCl	C. L. VII	0.70396	0.70397
		0.70398	
		0.70398	
C.			
0.01 n HCl + 0.09 n KCl	C. L. VIII 1	0.70408	
	C. L. VIII 2	0.70423	
	C. L. VIII 3	0.70399	

It will be seen from this (Section A, Table 1) that the agreement between the values newly arrived at and those previously found leaves nothing to be desired, save as regards the value for 0.01 n HCl, where, as the following comparison will show, a

slight difference appears between the value here found and that arrived at by Biilmann and Lund.

Experimental liquid	Biilmann Lund	Veibel	Biilmann Krarup	Sorensen Sorensen Lang	L.-Lang
0.01 n HCl	0.7044	—	—	0.7048	0.70471
0.02 n HCl	—	—	—	—	0.70464
0.05 n HCl	—	—	—	—	0.70456
0.10 n HCl	—	—	0.7044	—	0.70446
0.01 n HCl + 0.09 n KCl	—	0.7040	0.7042	—	0.70423

Fig. 2.



There seems, however, (see last column) to be a distinct drop in the value of  $E_{\text{KB}}$  when the concentration of hydrochloric acid in the experimental liquid is increased, and it is thus unlikely that  $E_{\text{KB}}$  should be the same in 0.01 n and 0.10 n HCl. On the contrary, the values found permit of a graphical extrapolation of  $E_{\text{KB}}^0$  as shown in Fig. 2. All measurements with HCl are here represented. The dark circles represent the mean values.

We obtain:

$$E_{KB} = -0.02885 \cdot \log k' = 0.70475 \pm 0.00005 \text{ (18}^\circ, 760 \text{ mm)}$$

from which  $E_{KB} = 0.70475 - 0.02885 \log \frac{f_H}{f_K}$  (2 a).

The figures in Table I show that there is a distinct difference between C. L. VII and C. L. VIII, the first product giving the potential value 0.70397, which agrees more or less with the value found previously by the writer and S. Kodama (l. c.), viz. 0.7038. That this value can hardly be due entirely to evaporation of the quinone is shown by Table I Section C, the products employed, C. L. VIII, 1, 2 and 3 being dried in the following manner:

C. L. VIII 1, dried for 3 days in open air.

— 2 — - 7 — between filter papers.  
— 3 — - 12 — — — —

The figures show that the quantity of quinone evaporated must be very slight, and the difference between C. L. VII and C. L. VIII may therefore be due to other causes.

Turning once more to the values for  $E_{KB}$  found by the use of C. L. VIII, our attention is drawn to the great difference between  $E_{KB}$  in 0.01 n HCl and 0.01 n HCl + 0.09 n KCl. The variation in potential with hydrochloric acid concentration  $c_{HCl}$  (0.01 n — 0.1 n) is no greater than we should expect from the determination of salting out of the hydroquinone and quinone in hydrochloric acid (cf. K. Linderstrøm-Lang l. c.), whereas the variation of  $E_{KB}$  with the potassium chloride concentration is considerably greater than we should expect.

Writing equation (1) as follows:

$$E_{KB} = -0.02885 \cdot \log k' - (0.02885 \cdot \log \frac{f_H}{f_K})_{0.01 \text{ n HCl}} - (0.02885 \cdot \log \frac{f_H}{f_K})_{0.09 \text{ n KCl}}$$

the sum of the first two terms on the right side of the sign is 0.70471, and the equation then appears as follows:

$$E_{KB} = 0.70471 - (0.02885 \cdot \log \frac{f_H}{f_K})_{0.09 \text{ n KCl}}$$

Solubility determinations (K. Linderstrøm-Lang l. c.) give the following expressions for the dependence of  $f_H$  and  $f_K$  on the potassium chloride concentration in solutions of potassium chloride in 0.01 n HCl:

$$\log f_H = 0.113 \cdot c_{KCl} \quad \log f_K = 0.023 \cdot c_{KCl},$$

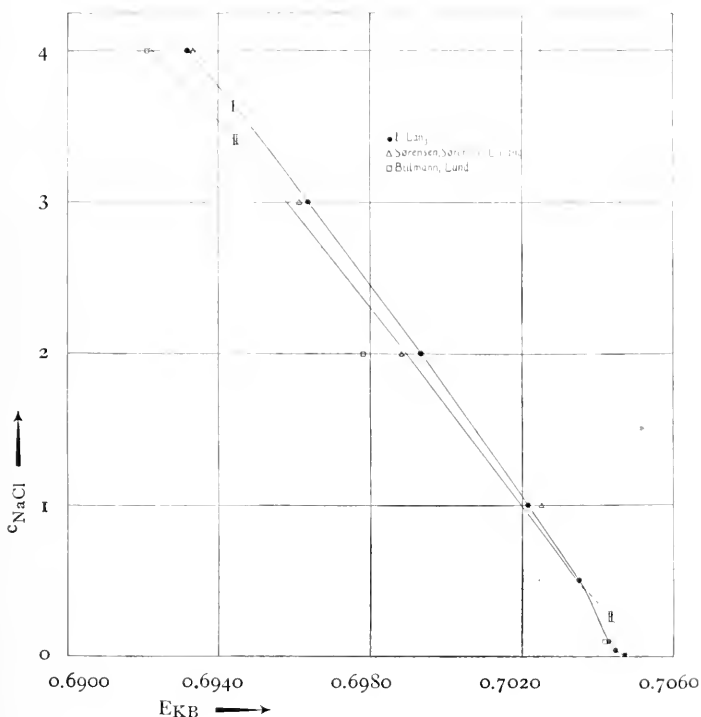
which by substitution gives:

$$E_{KB} = 0.70471 - 0.00023 = 0.70448,$$

a value differing from the 0.70425 by more than the experimental error.

The concentration of hydroquinone and quinone, however, as obtained by solubility determinations, is 0.5 and 0.1 molar respectively, whereas the corresponding concentration in the quinhydrone electrode is less than 0.02 molar, so that divergence on this account may be possible.

Fig. 3.



As regards the influence of NaCl on the value of  $E_{KB}$ , this has previously been investigated by Sørensen, Sørensen and Linderstrøm-Lang and Biilmann and Lund (l. c.) and shown in relation to the variation of solubility of hydroquinone and quinone in sodium chloride solutions, of varying concentration. As the agreement between the figures found at this laboratory and those arrived at by Biilmann and Lund leaves something to be desired, and as moreover, it would be interesting to see whether the influence of the sodium chloride at lower concentrations is of the same magnitude as that of the potassium chlo-

ride, I have once more determined the effect of the sodium chloride on the quinhydrone electrode, on  $E_{KB}$ . The result will be seen in Table 2, where also the potentials previously found are shown for purposes of comparison.

The agreement is not particularly good. As a cause of some of the discrepancies may be noted that in the apparatus in which  $E_{KB}$  was previously measured in this laboratory, there was a closed tap in the liquid connection between the two electrodes which might give rise to uncertainty in the potential measurement if the thin layer of liquid between the body of the tap and its socket were not perfectly homogeneous.

In Fig. 3,  $E_{KB}$  is set off as abscissa and the concentration of sodium chloride as ordinate. Curve I represents the measured values, Curve II those calculated from solubility determinations on the basis of the formula

$$E_{KB} = 0.70475 - 0.02885 \cdot \log \frac{f_H}{f_K}.$$

(Sorensen l. c.) as shown in Table 3.

Table 2.

$c_{HCl} + c_{NaCl}$	Biilmann Lund	Sorensen Sorensen L-Lang	L-Lang	L-Lang mean
0.01 + 0.00	0.7044	0.7048	0.70471	0.70471
0.01 + 0.04	—	—	0.70448 0.70446	0.70447
0.01 + 0.09	0.7042	—	0.70425 0.70431	0.70428
0.01 + 0.49	—	0.7041	0.70348 0.70349 0.70356	0.70351
0.01 + 0.99	—	0.7025	0.70215 0.70213	0.70214
0.01 + 1.99	0.6978	0.6988	0.69927 0.69940	0.69934
0.01 + 2.99	—	0.6961	0.69643 0.69629	0.69636
0.01 + 3.99	0.6921	0.6933	0.69321 0.69319	0.69320



Table 3.

$c_{\text{HCl}} + c_{\text{NaCl}}$	$0.02885 \cdot \log \frac{f_{\text{H}}}{f_{\text{K}}}$	$E_{\text{KB}}$ (calculated)
0.00 + 0.00	0	0.70475
0.01 + 0.00	0.00001	0.70474
0.01 + 0.09	0.00000	0.70475
0.01 + 0.49	0.00130	0.70345
0.01 + 0.99	0.00276	0.70199
0.01 + 1.99	0.00579	0.69896
0.01 + 2.99	0.00894	0.69581
0.01 + 3.99	0.01252	0.69223

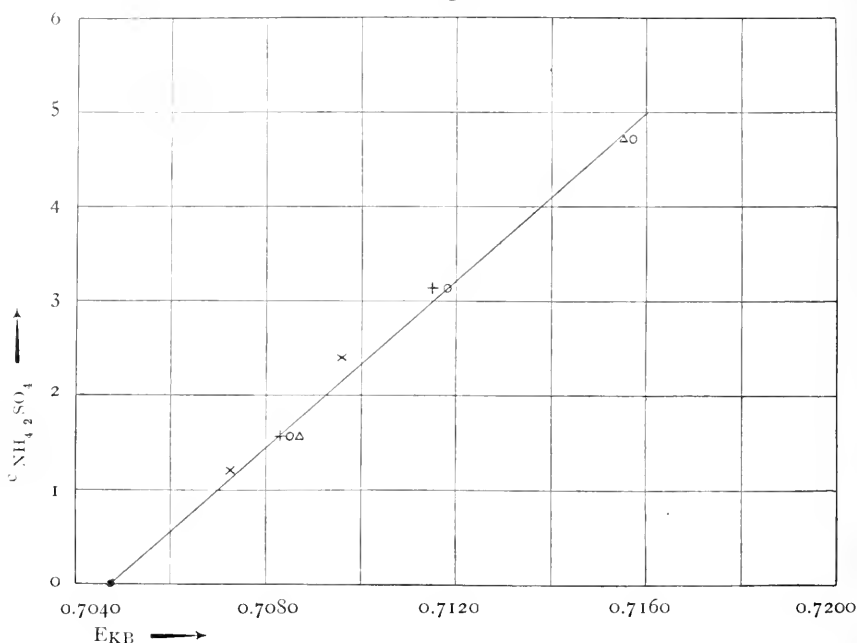
We find here, in the first place, that the alteration in potential at the lower concentrations of sodium chloride takes a course differing from that of the rest of the curve, and that the curve found here differs greatly from that calculated. In other words, sodium chloride behaves as does potassium chloride, the variation of  $E_{\text{KB}}$  with the concentration in dilute solutions being in both cases greater than that calculated from the solubility measurements. These are, however, not exact when the salt concentration is slight, and the divergence can therefore probably be explained as due to experimental errors. As regards the remainder of the curve, the difference from the calculated curve increases with the salt concentration. This is doubtless due to the feature noted on p. 9, that the values of the hydroquinone and quinone concentrations differ greatly in solubility measurement and potential measurement.

Why the potential curve should have this peculiar shape it is difficult to say from these few experiments.

## B. The Ammonium Sulphate Error.

The measurements of  $E_{\text{KB}}$  with ammonium sulphate solutions as experimental liquid cannot be as accurate as those previously mentioned. For the slight trace of ammonia always present in such solutions will react upon the quinone, presumably in the same way as ammonia and amines combine with formaldehyde. Red to blue colouring matter is here formed with great intensity. That it is the free ammonia which reacts is seen from the fact that the reaction velocity depends on the concentration of hydrogen ions, and increases when this is reduced.

Fig. 4.



As the concentration of hydrogen ions in the protein solutions which require correction for the ammonium sulphate error in measurement often lies about  $p_H = 4.7$ , I have regulated the hydrogen ion concentration in ammonium sulphate solutions by means of 0.01 sodium acetate-acetic acid mixtures to the  $p_H$  values 5, 4.5 and 4, and the process mentioned here takes place comparatively rapidly. The variation in potential thus occasioned is however, so slight that we can, with an accuracy of 0.3 to 0.4 millivolt, take the mean value of the potentials measured in the course of the first ten minutes after adding the quinhydrone, and curiously enough, the potential will often remain constant for a considerable time.

Table 4 gives the measurements, and Fig. 4  $E_{KB}$  as abscissa, with the ammonium sulphate concentration (normality) as ordinate.

It will be noticed that the ammonium sulphate acts in a manner entirely opposite to that of the sodium chloride. As to the cause of this difference, I will refer the reader to a subsequent work on the relation between ionic magnitude and salting out effect, and will here only mention a few solubility experiments with hydroquinone, quinone and ammonium sulphate solutions, which serve to show the agreement between the  $E_{KB}$  thus calculated and that found directly.

Table 4.

$c_{(\text{NH}_4)_2\text{SO}_4}$	$p_{\text{H}}$ ca.	$E_{\text{KB}} (18^\circ)$ dry hydrogen	Mrk. on Curve Fig. 4.
1.569 n	4.48	0.7085	O
3.138	—	0.7118	
4.707	—	0.7157	
1.569	4.95	0.7087	$\Delta$
4.707	—	0.7155	
1.569	4.03	0.7083	+
3.138	—	0.7115	

For the manner in which these experiments were carried out, see K. Linderström-Lang<sup>1)</sup>. The temperature was  $18^\circ$ . If  $s_0$  be the solubility in 0.01 n HCl,  $s_n$  in ammonium sulphate solution, then  $f = s_0/s_n$ . The results will be seen in Table 5.

Table 5.

$c_{(\text{NH}_4)_2\text{SO}_4}$	Hydroquinone			$s$	Quinone	
	$s$	$f$	$\log f$		$f$	$\log f$
0 (0.01 n HCl)	0.5077	1.000	0.0000	0.1013	1.000	0.0000
1.2 n	0.4030	1.260	0.1004	0.0656	1.544	0.1886
2.4	0.3167	1.603	0.2049	0.0428	2.367	0.3742

$c_{(\text{NH}_4)_2\text{SO}_4}$	$\log f_{\text{H}} - \log f_{\text{K}}$	$0.02885 \log \frac{f_{\text{H}}}{f_{\text{K}}}$	$E_{\text{KB}}$ (calculated)
0 (0.01 n HCl)			0.70471
1.2 n	— 0.0882	— 0.00254	0.70725
2.4	— 0.1693	— 0.00488	0.70959

It will be seen from Fig. 4 where the calculated values are marked by  $\times$ , that there is a slight difference between the calculated curve and that found for alteration in potential, a difference tending in the same direction as in the case of the sodium chloride.

### C. The Protein Error.

Determination of the protein error is in some cases attended by considerable difficulty. Acid casein solutions and egg albumin solutions can be measured fairly easily, and give constant poten-

<sup>1)</sup> Compt.-rendus du Lab. Carlsb. **15**, No 4 (1923).

tials, whereas the solutions of serum albumin which I have had to work with were difficult to measure in this respect. They were highly viscous, opalescent, contained ammonium sulphate, and easily poisoned the electrodes, especially, it would seem, the hydrogen electrode, and they gave potentials varying at best 0.3 millivolt in ten minutes, at worst 1 millivolt during the same period, though the hydrogen ion concentration was about  $p_H = 4.7$ . I have only used those measurements where the alteration in the first ten minutes after adding the quinhydrone was less than 0.5 millivolt; but the difference between results of parallel experiments may nevertheless amount to 1.3 millivolts. The determination of the protein error cannot therefore in this case be effected with any high degree of accuracy.

As the protein error appears to vary considerably with the character of the protein used, the following measurements cannot be used for correction of measurements with the quinhydrone electrode in protein solutions generally, but only serve to give an idea as to the order of magnitude of the errors to be reckoned with.

In the following tables,  $c_N$  denotes the no. of gramme equivalents protein nitrogen,  $c_{NH_4^2SO_4}$ ,  $c_{HCl}$ ,  $c_{NaOH}$  the no. of ammonium sulphate, hydrochloric acid, and sodium hydroxide equivalents respectively per litre of protein solution.  $E_p$  is the  $E_{KB}$  measured with the protein solution as experimental liquid, while  $E_{AM}$  is the potential when the protein solution is replaced by a pure ammonium sulphate solution of the same normality.  $E_{AM}$  can be found from the curve in Fig. 4, when the ammonium sulphate concentration of the protein solution is known.

Table 6.

## Casein measurements.

$c_{NaCl}$	$c_{HCl}$	$c_{NaOH}$	$p_H$	$E_p$	Remarks
0	0	0.03	ca. 7	0.7043	E measured directly after adding the quinhydrone
0	0	0.03	7	0.7043	
0.015	0.025	0	2.24	0.7044	E constant
0.015	0.025	0	2.24	0.7045	do.
0.028	0.006	0	3.53	0.7044	do.

$$c_N = 0.55$$

Table 7.

Serum albumin ( $M_5$ )

$c_{(NH_4)_2SO_4}$	$c_N$	$c_{HCl}$	$c_{NaOH}$	$p_{aH}$	$E_p$	$E_p$ (mean)
0.416 n	0.275	0	0	4.7	0.7069	0.7069
0.416	0.275	0	0	4.7	0.7068	
0.500	0.330	0	0	4.7	0.7078	0.7078
0.500	0.330	0	0	4.7	0.7078	
0.833	0.550	0	0	4.7	0.7085	0.7087
—	—	-	-	—	0.7088	
—	—	-	-	—	0.7091	
—	—	-	-	—	0.7083	
—	—	-	-	—	0.7079	
—	—	-	-	—	0.7093	
—	—	-	-	—	0.7092	
—	—	-	-	—	0.7087	
1.665	1.100	0	0	4.7	0.7121	0.7122
—	—	-	-	—	0.7122	
1.928	0.330	0	0	4.7	0.7112	0.7112
2.396	0.236	0	0	4.7	0.7115	0.7115
0.500	0.330	0	0.006	5.44	0.7079	0.7079
0.500	0.330	0.006	0	4.35	0.7085	0.7085
0.416	0.275	0.01	0	4.2	0.7081	0.7080
—	—	-	-	—	0.7079	
0.833	0.550	0.02	0	4.2	0.7093	0.7094
—	—	-	-	—	0.7088	
—	—	-	-	—	0.7100	
0.500	0.330	0.012	0	4.01	0.7089	0.7089

Table 8.

Serum albumin, ( $M_5$ ). Dependence of protein error on concentration of protein.

$c_{(NH_4)_2SO_4}$	$c_N$	$p_{aH}$	$E_P$	$E_{AM}$	$E_P - E_{AM}$
2.396 n	0.236	4.7	0.7115	0.7101	+0.0014
0.416	0.275	—	0.7069	0.7057	0.0012
0.500	0.330	—	0.7078	0.7059	0.0019
1.928	0.330	—	0.7112	0.7091	0.0021
0.833	0.550	—	0.7087	0.7066	0.0021
1.665	1.100	—	0.7122	0.7085	0.0037

Table 9.

Serum albumin, ( $M_5$ ). Dependence of protein error on concentration of hydrogen ions.

$c_{(NH_4)_2SO_4}$	$c_N$	$p_{aH}$	$E_P$	$E_{AM}$	$E_P - E_{AM}$	$(E_P - E_{AM})/c_N$
0.500 n	0.330	4.01	0.7089	0.7059	0.0030	0.0091
0.416	0.275	4.2	0.7080	0.7057	0.0022	0.0080
0.500	0.330	4.35	0.7085	0.7059	0.0026	0.0078
0.500	0.330	4.7	0.7078	0.7059	0.0019	0.0058
0.416	0.275	4.7	0.7069	0.7057	0.0012	0.0044
0.500	0.330	5.44	0.7079	0.7059	0.0020	0.0061

Table 10.

Egg albumin, (D. Æ. 5).

$c_{(NH_4)_2SO_4}$	$c_N$	$c_{HCl}$	$p_{aH}$	$E_P$
0.016 n	0.288	0	5.27	0.7033
—	—	0.004	4.79	0.7034
—	—	0.008	4.49	0.7040
—	—	0.012	4.23	0.7044
1.442	—	0	5.41	0.7062
1.311	0.262	0.012	4.23	0.7074

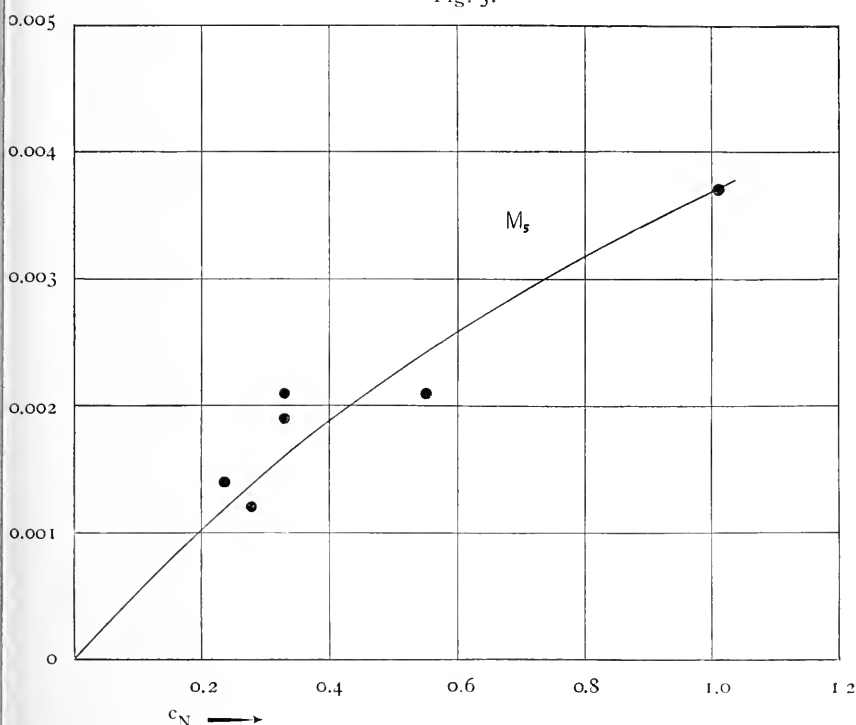
Assuming now that the salt effect and the protein effect in a saline protein solution enter into additive combination, then  $E_P - E_{AM}$  will be a measure of the influence of the protein alone, and should, therefore, at a constant protein concentration, be independent of the salt concentration, which, from Table 8 and Table 11, appears to be approximately correct.

Tabel 11.

Egg albumin, (D. Æ. 5). Dependence of protein error on the concentration of hydrogen ions.

$c_{(\text{NH}_4)_2\text{SO}_4}$	$c_N$	$\text{pH}$	$E_P$	$E_{AM}$	$E_P - E_{AM}$	$(E_P - E_{AM})/c_N$
1.442 n	0.288	5.41	0.7062	0.7081	-0.0019	-0.0066
0.016	0.288	5.27	0.7033	0.7048	-0.0015	-0.0052
0.016	0.288	4.79	0.7034	0.7048	-0.0014	-0.0049
0.016	0.288	4.49	0.7040	0.7048	-0.0008	-0.0028
0.016	0.288	4.23	0.7044	0.7048	-0.0004	-0.0014
1.311	0.262	4.23	0.7074	0.7077	-0.0003	-0.0016

Fig. 5.

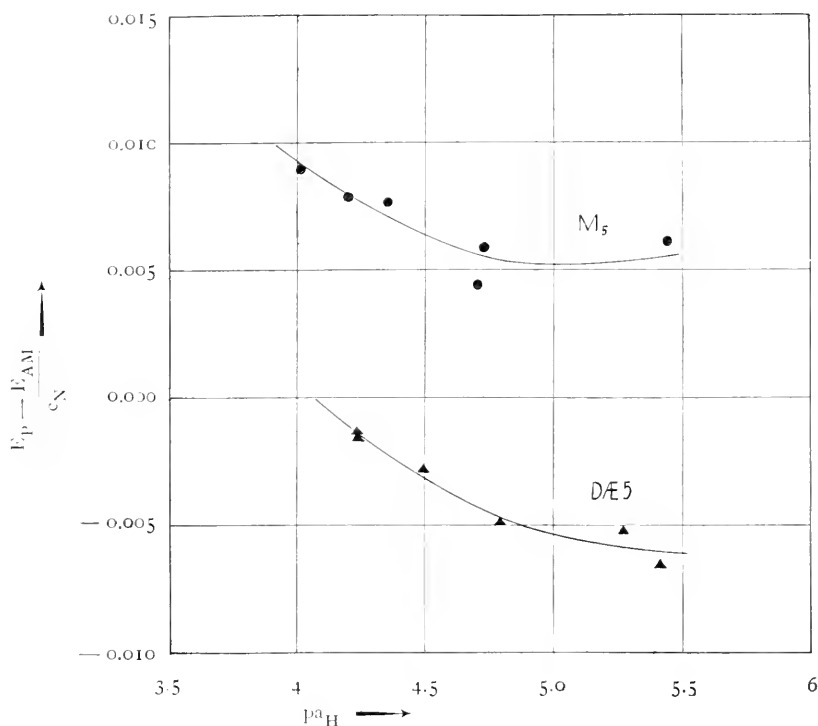


The alteration in potential is as a rule proportional to the concentration of the substance producing it. This will be seen in Fig. 3 and Fig. 4, and is due to the fact that the term  $0.0285 \log (f_H/f_K)$  varies approximately lineally with the concentration (see Linderström-Lang l. c.). The quantity  $(E_P - E_{AM})/c_N$  can therefore be used with a fair degree of accuracy (see Fig. 5)

for comparing the effect at two different protein concentrations not very far apart. (See Tables 9 and 11).

It will be seen from the tables, and still more distinctly from Figs. 5 and 6, how a protein content in the experimental

Fig. 6.



liquid affects the potential of the quinhydrone electrode in regard to the hydrogen electrode. Table 6 shows that casein has an effect just on the verge of the error of measurement, and that this effect is independent of the concentration of hydrogen ions. In alkaline casein solutions, the quinhydrone electrode does not give constant potentials, and  $E_P$  was therefore measured immediately after adding the quinhydrone. In the acid casein solutions on the other hand, the potential is nicely constant.

As regards the serum albumin ( $M_5$ ), the alteration of potential with the protein concentration (Fig. 5) is here considerably greater, and tends in the same direction as the ammonium sulphate error; there is also a slight, but distinct alteration with the concentration of hydrogen ions. (Fig. 6).



The egg albumin error is likewise dependent on the concentration of hydrogen ions, but with opposite sign to that of the serum albumin error, and is somewhat less. (Fig. 6).

The alteration in the albumin error with concentration of hydrogen ions runs more or less parallel in both albumins (Fig. 6). I shall endeavour to explain these features in a later publication.

#### D. Application of the Results Above Noted in Determination of Hydrogen Ions.

(Correction of  $p_H$  and  $pa_H$  for electrolyte and protein errors)

In a previous work by S. P. L. Sorensen and the present writer<sup>1)</sup> the following formulæ were given for determination both of  $p_H$  and  $pa_H = -\log a_H$ , by means of the hydrogen electrode:

$$p_H = \frac{E_{H_2} - 0.3380}{0.0577} (18^\circ, 760 \text{ mm. dry hydrogen}) \quad (3)$$

$$pa_H = \frac{E_{H_2} - 0.3357}{0.0577} \quad \text{do.} \quad (4)$$

$E_{H_2}$  being the potential (corr. for liquid junction potential) of the element:

Calomel electr. 0.1 n KCl/3.5 n KCl/Experimental liq.  $H_2$ , Pt.  
(18°, 760 mm)

Calling the potential (corr.) of the element:

Pt. quinhydrone, exper. liq./3.5 n KCl/0.1 n KCl-calomel electr.

$E_{Kin}$ , then according to equation (2 a) p. 8.

$$E_{KB} = E_{Kin} + E_{H_2} = 0.70475 - 0.02885 \log \frac{f_H}{f_K}$$

an expression which inserted in (3) and (4) gives:

$$p_H = \frac{0.36675 - E_{Kin}}{0.0577} + \left( -0.5 \log \frac{f_H}{f_K} \right)^2 \quad (5)$$

$$pa_H = \frac{0.36905 - E_{Kin}}{0.0577} + \left( -0.5 \log \frac{f_H}{f_K} \right)^2 \quad (6)$$

The last equations apply to any experimental liquids, and are transformed into:

<sup>1)</sup> Comptes-rendus du Lab. Carlsberg. **15**, No 6 (1924).

<sup>2)</sup> These formulæ agree entirely with the standardisation proposals put forward in the paper above quoted, p. 39, section 8.

$$p_H = \frac{0.36675 - E_{K_{in}}}{0.0577} \quad (7)$$

$$p_{aH} = \frac{0.36905 - E_{K_{in}}}{0.0577} \quad (8)$$

when the experimental liquid is very highly diluted.

The last term on the right side of (5) and (6) is a correction for the error arising from the salt, protein or other substance in the experimental liquid. This correction term we will call  $Q$ , bearing in mind that it is given by the expression:

$$Q = \frac{E_{KB} - E_{KB}^0}{0.0577} = \frac{E_{KB} - 0.70475}{0.0577} \quad (9)$$

From this formula, with the values and curves given in the previous section for  $E_{KB}$  in ammonium sulphate, hydrochloric acid, potassium chloride, sodium chloride solutions, the following tables for  $Q$  have been calculated. From these,  $Q$  can be found, and  $p_H$  or  $p_{aH}$  can be calculated from the equations:

$$p_H = \frac{0.36675 - E_{K_{in}}}{0.0577} + Q \quad (10)$$

$$p_{aH} = \frac{0.36905 - E_{K_{in}}}{0.0577} + Q \quad (11)$$

As regards the protein solutions, the correction for ammonium sulphate content and the correction for protein content are taken together:

$$Q = \frac{E_P - 0.70475}{0.0577} = Q_{AM} + Q_P = \frac{E_{AM} - 0.70475}{0.0577} + \frac{E_P^0 - 0.70475}{0.0577},$$

where  $E_P^0$  is the potential in a protein solution free from salt. From this we obtain:

$$Q_P = \frac{E_P^0 - 0.70475}{0.0577} = \frac{E_P - E_{AM}}{0.0577}$$

(See also p. 16).

Table 12.

Correction for hydrochloric acid.

$c_{\text{HCl}}$	$E_{\text{KB}} - E_{\text{KB}}^0$	$Q_{\text{HCl}}$
0.01	- 0.00006	- 0.001
0.02	- 0.00010	- 0.002
0.05	- 0.00019	- 0.003
0.10	- 0.00029	- 0.005

For the experimental liquid 0.01 n HCl + 0.09 n KCl,  $Q = - 0.000$ .

Table 13.

Correction for NaCl in 0.01 n HCl.

$c_{\text{NaCl}}$	$E_{\text{KB}} - E_{\text{KB}}^0$	$Q_{\text{NaCl}}$
0.04	- 0.00028	- 0.005
0.09	- 0.00047	- 0.008
0.49	- 0.00124	- 0.021
0.99	- 0.00261	- 0.045
1.99	- 0.00541	- 0.094
2.99	- 0.00839	- 0.145
3.99	- 0.01155	- 0.200

Table 14.

Correction for  $(\text{NH}_4)_2\text{SO}_4$ . Read from curve Fig. 4.

$c_{(\text{NH}_4)_2\text{SO}_4}$	$E_{\text{KB}} - E_{\text{KB}}^0$	$Q_{\text{AM}}$
0.5	+ 0.0011	+ 0.019
1.0	0.0022	0.038
1.5	0.0033	0.057
2.0	0.0045	0.078
2.5	0.0056	0.097
3.0	0.0067	0.116
3.5	0.0078	0.135
4.0	0.0090	0.156
4.5	0.0101	0.175
5.0	0.0112	0.194

Table 15.

Correction for egg albumin. Read from curve Fig. 6.

$c_N$	$p_{aH}$	$(E_P - E_{AM})/c_N$	$E_P - E_{AM}$	$Q_P$	$Q_P/c_N$
0.3	4.0	+ 0.0007	+ 0.0002	+ 0.003	+ 0.010
—	4.5	— 0.0032	— 0.0010	— 0.017	— 0.057
—	5.0	— 0.0054	— 0.0016	— 0.028	— 0.093
—	5.5	— 0.0061	— 0.0018	— 0.031	— 0.103

Table 16.

Correction for serum albumin (varying hydrogen ion activity).

Read from curve Fig. 6.

$c_N$	$p_{aH}$	$(E_P - E_{AM})/c_N$	$E_P - E_{AM}$	$Q_P$	$Q_P/c_N$
0.3	4.0	+ 0.0093	+ 0.0028	+ 0.048	+ 0.160
—	4.5	0.0064	0.0019	0.033	0.110
—	5.0	0.0052	0.0016	0.028	0.093
—	5.5	0.0057	0.0017	0.029	0.097

Table 17.

Correction for serum albumin (varying  $c_N$ ).

Read from curve Fig. 5.

$c_N$	$p_{aH}$	$E_P - E_{AM}$	$Q_P$
0.1	4.7	+ 0.0005	+ 0.009
0.2	—	0.0010	0.017
0.4	—	0.0019	0.033
0.6	—	0.0026	0.045
0.8	—	0.0032	0.055
1.0	—	0.0037	0.064

Casein: greatest  $Q = - 0.008$ .

As an example of the use of these tables we may take the following: In electrometric measurement of a solution of serum albumin ( $M_5$ ) with ammonium sulphate concentration 1.57 n, and protein concentration  $c_N$  0.65, the potential  $E_{Kin} = 0.0992$  has been determined by means of the quinhydrone electrode.  $p_{aH}$  is therefore given by (11):

$$p_{aH} = \frac{0.3691 - 0.0992}{0.0577} + Q = 4.678 + Q$$

From Table 14 we now find, first  $Q_{AM} = +0.060$  at the ammonium sulphate concentration 1.57*n*. Then from Table 17,  $Q_P = +0.048$  at the hydrogen ion concentration abt. 4.7, when the above mentioned  $Q$  is calculated from:

$$Q = Q_P + Q_{AM} = 0.104.$$

and  $p_{H^+}$  is then:

$$4.678 + 0.104 = 4.782.$$

Where the hydrogen ion activity of the solution does not lie about 4.7, we must get out the value for  $Q_P/c_N$  from Table 16, at the preliminary uncorrected  $p_{H^+}$  value, and multiply by the protein concentration  $c_N$ . But the value for  $Q_P$  thus obtained is somewhat incorrect.

The figures found for protein correction can hardly be compared with previous measurements by Kolthoff (l. c.) and V. Lester<sup>1)</sup>, as these writers worked with natural solutions of proteins such as blood serum and milk.

In blood serum,  $c_N$  is abt. 0.8 and in milk abt. 0.4. We find:

Kolthoff: Blood serum:	$p_H = 3.8$	$Q = -0.04$
	5.3	$Q = -0.03$
	5.9	$Q = +0.15$
Kolthoff: Milk:	$p_H = 6.7$	$Q = +0.09$
	Lester: Milk: 6.7	$Q = +0.04$

which agree but poorly with the values given above. Kolthoff has also a series of measurements with sodium caseinate solutions of  $p_H =$  abt. 7, where he finds  $Q$  values up to + 0.23. Unfortunately, the casein concentration is not stated, but it must be regarded as likely that the difference lies in the method of measurement, as Kolthoff, from what I can see, waits longer than I have done after adding the quinhydrone before taking the measurement. Something of the same sort may perhaps apply to the other differences noted, and it should be pointed out that the direct measurement of  $E_{KB}$  must on principle be regarded as the most accurate.

## E. Summary.

1. In potential measurement of the element:

(1) Pt., quinhydr., exper. liquid.  $H_2$  (1 atmos. dry hydrogen) Pt. (18<sup>0</sup>) using hydrochloric acid solutions of varying slight con-

<sup>1)</sup> Journ. Agricult. Science **14**, (4), 634, (1924).

centration. the potential of (I)  $E_{KB}^0$  with pure water as experimental liquid is extrapolated to:

$$E_{KB}^0 = 0.70475 \pm 0.00005 \text{ Volt.}$$

2. The lack of agreement between previous measurements of this potential is due chiefly to the fact that it has been identified with the potential  $E_{KB}$  of (I) with the experimental liquids 0.01 n HCl, 0.1 n HCl and 0.01 n HCl + 0.09 n KCl, as it has been found that there is a considerable electrolyte effect on the quinhydrone in these dilute electrolyte solutions.

3. The alteration in  $E_{KB}$  on adding sodium chloride to the experimental liquid 0.01 n HCl, — concentration 0.04 to 3.99 — has been determined anew, and again brought into relation with the salting out of the quinone and hydroquinone by sodium chloride.

4. The alteration in  $E_{KB}$  with the concentration of ammonium sulphate in the experimental liquid has been determined, and found to tend in the opposite direction to that of the alteration in sodium chloride solutions.

5. The alteration of  $E_{KB}$  with the concentration of certain proteins, casein, egg albumin and serum albumin, has been measured and found to depend on the nature of the protein; in the case of the last two, also on the concentration of hydrogen ions.

6. The following formulæ are given for calculation of  $p_H$  and  $p_{aH}$  from measurements with the quinhydrone electrode:

$$p_H = \frac{0.36675 - E_{Kin}}{0.0577} + Q$$

$$p_{aH} = \frac{0.36905 - E_{Kin}}{0.0577} + Q$$

where  $E_{Kin}$  is the potential (corr. for liquid junction potential) of the element:

Pt, quinhydr., exper. liquid/3.5 n KCl/0.1 n KCl calomel electr. 18°, and  $Q$  is the correction for the influence of dissolved substances in the experimental liquid.

7. The  $Q$  values for the experimental liquids here considered are presented in tabular form.

In conclusion, I wish to express my very sincere thanks to Prof. S. P. L. Sørensen for kindly inviting me to undertake these measurements, and for his valuable criticism in the course of the work.

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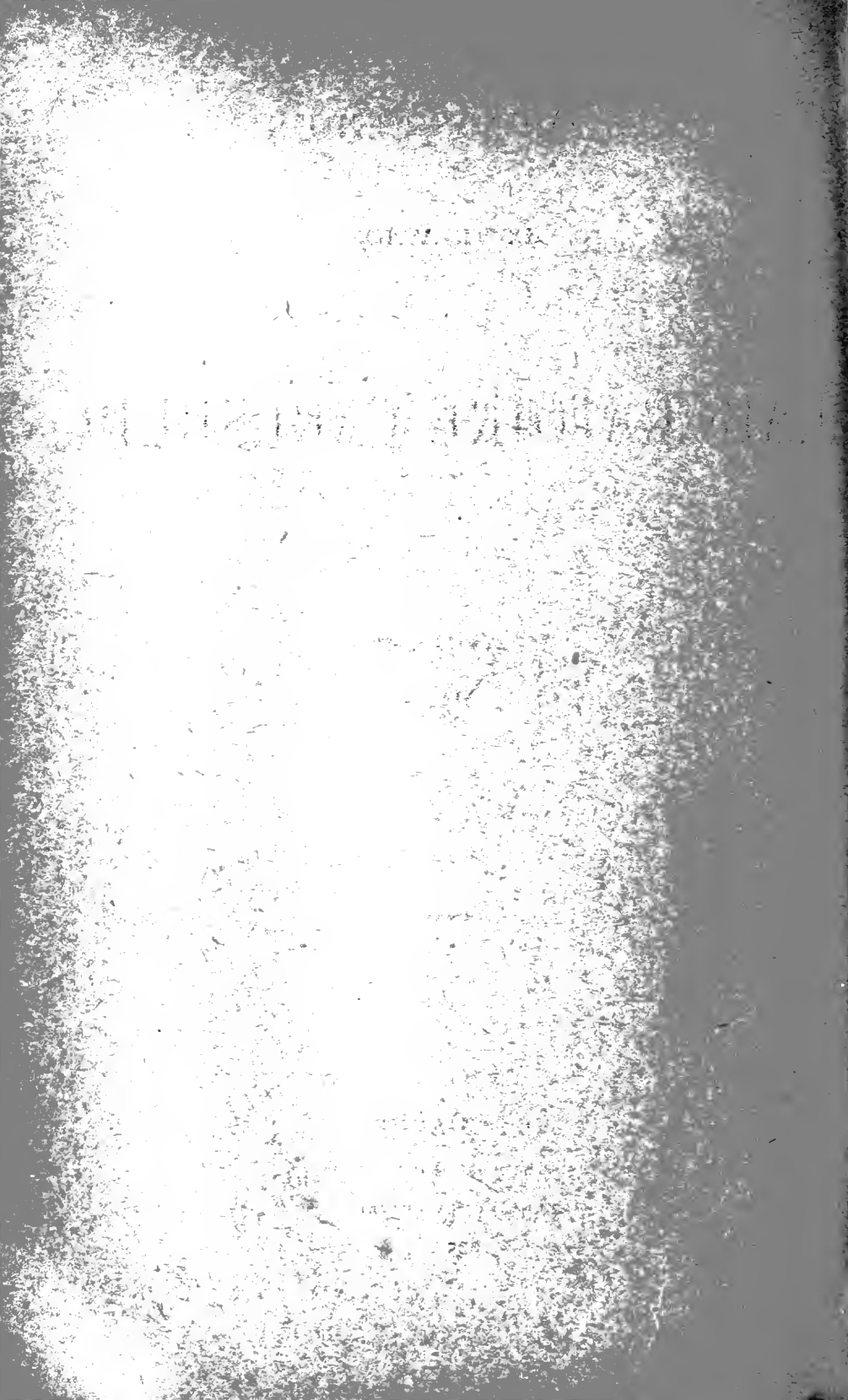
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# SUR LE DOSAGE DES MÉLANGES DE SACCHAROSE ET DE SUCRE INTERVERTI OU DE LACTOSE. II.

PAR

HANS JESSEN-HANSEN.

Dans les tables précédemment publiées<sup>1)</sup> pour l'analyse de ces mélanges, le sucre de canne ou interverti sont supposés n'exister qu'en proportion inférieure ou, au plus, égale à quatre fois celle du sucre de lait. Maintenant, cependant, la fabrication fournit couramment des produits à base de lait condensé auquel on a ajouté des doses de sucre de canne notablement plus considérables et, par conséquent, lors de l'examen de ces produits, les tables ci-dessus citées se trouvent mises en défaut. Aussi, les chimistes s'occupant de ce genre de recherches ont-ils exprimé le désir de voir élargir ces tables, désir auquel le Laboratoire Carlsberg a crû devoir répondre de son mieux. C'est ce qui a provoqué l'apparition du présent travail, qui permettra de faire l'analyse de mélanges contenant jusqu'à 8—9 fois autant de saccharose que de lactose.

En outre, on a émis le soupçon que l'intervention d'après Nicol<sup>2)</sup>, prescrite dans notre mémoire précédent, ne laisse pas inaltéré le lactose, contrairement à ce que nous avons supposé en établissant la Table V, relative au dosage des mélanges de sucre de canne et de lait; par suite, les données contenues dans cette table ne seraient pas tout à fait correctes.

Il faut bien reconnaître que sur ce dernier point on n'a pas tort. En effet, lorsqu'on soumet au traitement en question une solution de lactose, c'est-à-dire qu'on lui fait subir le chauffage pendant une demi-heure dans l'eau bouillante après avoir ajouté

<sup>1)</sup> Ces mêmes comptes rendus, Vol. XV, n° 3 (1923).

<sup>2)</sup> Zeitschrift für an. Chemie XIV, 177 (1875).

à 70 cm<sup>3</sup> de la solution environ 2 cm<sup>3</sup> d'acide chlorhydrique normal, on observera que le pouvoir réducteur ne sera pas resté tout à fait inchangé. L'essai que nous allons relater le fera voir.

On fit dissoudre 1 gramme de lactose dans 50 cm<sup>3</sup> d'eau + 20 cm<sup>3</sup> de HCl, norm. au 1/10; la solution ainsi obtenue fut chauffée pendant une demi-heure dans l'eau bouillante, puis refroidie, neutralisée avec 20 cm<sup>3</sup> NaOH norm. au 1/10 et complétée à 100 cm<sup>3</sup> = 100<sup>gr</sup>,3033. Le pouvoir réducteur de quelques quantités pesées de cette solution fut déterminé, puis comparé avec la Table III (détermination de lactose pur<sup>1)</sup>).

Les résultats de ces expériences se trouvent inscrits au tableau I, qui fait voir que le pouvoir réducteur du lactose s'est accru de 1.5 % à la suite du traitement par un acide en vue de l'intervention d'après Nicol.

Tableau I.  
Lactose traité d'après Nicol.

Lactose	Cu		Diff.	
	Trouvé	Table III	F ÷ Table III	Diff. en %
23.4 .....	33.4	32.3	+ 1.1	+ 3.4
25.4 .....	32.1	32.3	÷ 0.2	÷ 0.6
50.15 .....	63.7	63.4	+ 0.3	+ 0.5
50.2 .....	65.0	63.5	+ 1.5	2.4
99.2 .....	127.2	125.0	+ 2.2	1.7
149.8 .....	190.8	188.2	+ 2.6	1.4
198.5 .....	252.5	249.2	+ 3.2	1.3
198.8 .....	252.3	249.4	+ 2.9	1.2
249.0 .....	317.3	312.3	+ 5.0	1.6
250.0 .....	317.4	313.4	+ 4.0	1.3
298.9 .....	381.4	374.5	+ 6.9	1.8
				+ 1.5 %

Vu que le traitement par acide nécessaire pour l'inversion du saccharose s'est ainsi trouvé exercer une faible influence sur le lactose présent, il est évident qu'il fallait en tenir compte en élaborant une nouvelle table, laquelle devait du reste être dressée suivant le même principe que l'ancienne table V. Pour cela, nous avons procédé comme il suit:

<sup>1)</sup> *loc. cit.*

1°. 187,000 de lactose fut dissous dans 50 cm<sup>3</sup> d'eau + 20 cm<sup>3</sup> de HCl norm. au 1/10.

2°. 087,950 de saccharose, de même.

Les deux solutions furent chauffées une demi-heure dans l'eau bouillante, puis refroidies à la température ambiante et neutralisées par 20 cm<sup>3</sup> NaOH norm. au 1/10, après quoi on compléta la 1<sup>ière</sup> solution à exactement 500 cm<sup>3</sup>, tandis que la 2<sup>e</sup> fut complétée à 100 cm<sup>3</sup>, puis pesée. Ainsi, 1 cm<sup>3</sup> de la solution 1° contient 2 mgr. de lactose soumis au même traitement par acide que le saccharose interverti, alors que la solution 2° renferme 10 mgr de sucre interverti par centimètre cube. Ensuite, à «50 cm<sup>3</sup> Fehling» (c'est-à-dire 8<sup>gr</sup>.65 sel de Seignette, 25 cm<sup>3</sup> NaOH 3.25 normal et 25 cm<sup>3</sup> CuSO<sub>4</sub> 0.277 mol.), on a ajouté de la manière habituelle 12.5 cm<sup>3</sup> de la solution 1° = 25 mgr. de lactose. et l'on y a ajouté, en pesant, une dose convenable de la solution de sucre interverti. Cela fait, on compléta à 100 cm<sup>3</sup> avec de l'eau, on chauffa dans une atmosphère d'hydrogène pendant, bien exactement, 5 minutes dans de l'eau bouillante. Enfin, après filtration à chaud, l'oxyde de cuivre qui s'était séparé, fut lavé, réduit dans un courant d'hydrogène et pesé.

Au moyen des résultats obtenus par un nombre suffisant de ces essais, nous avons calculé l'équation habituelle. Elle s'est trouvée être :

$$\text{Cu} = 32.02 + 1.9265 \text{ I} \div 0.0005794 \text{ l}^2.$$

Dans le tableau 2 se trouvent groupées les valeurs numériques correspondantes, obtenues par les essais ci-dessus relatés, du sucre interverti et du cuivre, ainsi que les valeurs du cuivre calculées à l'aide de l'équation ci-dessus. — Quant aux essais mis entre parenthèses, ils ont été exécutés postérieurement au calcul de cette équation; on verra que leurs résultats concordent parfaitement avec ceux des autres essais.

La table finale, VI, contient les proportions de sucre interverti qui, d'après l'équation ci-dessus, correspondent à tous les milligrammes entiers de cuivre. En ce qui concerne l'utilisation pratique de cette table (en vue de recherches analytiques), nous renvoyons aux explications données dans notre premier mémoire.

Inutile de faire observer que le fait susmentionné que le traitement par acide nécessaire pour l'inversion du saccharose ne

Tableau 2.

0.025 lactose + sucre interverti.

$$\text{Cu} = 32.02 + 1.92650 \text{ I} \div 0.0005794 \text{ I}^2.$$

Sucre interverti	Cu		Diff. T $\div$ C
	Trouvé	Calculé	
0 .....	32.2	32.02	+ 0.18
0 .....	32.7	32.02	+ 0.68
(24.60.....	78.8	79.06	$\div$ 0.26)
27.26.....	84.1	84.11	$\div$ 0.01
39.30.....	107.1	186.82	+ 0.38
(47.05.....	121.1	121.38	$\div$ 0.28)
49.81.....	125.9	126.54	$\div$ 0.64
52.91.....	131.2	132.47	$\div$ 1.27
(93.46.....	207.0	207.01	$\div$ 0.01)
99.78.....	219.4	218.48	+ 0.92
100.27.....	219.3	219.36	$\div$ 0.06
(141.90.....	294.5	293.72	+ 0.78)
149.71.....	307.3	308.45	$\div$ 1.15
150.77.....	309.8	309.31	+ 0.49
162.72.....	330.2	330.16	+ 0.04
175.43.....	352.4	352.16	+ 0.24
(187.60.....	373.1	373.03	+ 0.07)
199.60.....	392.0	393.47	$\div$ 1.47
201.19.....	396.8	396.16	+ 0.62
(221.10.....	427.6	429.00	$\div$ 1.40)
(219.84.....	427.7	427.54	+ 0.16)

laisse pas inaltéré le lactose, a pour conséquence que l'ancienne Table V est entachée d'une erreur systématique. Puisque la nouvelle Table VI peut être utilisée dans tous les cas où l'on pourrait faire usage de la Table V, il est à présumer que cette dernière cessera d'être employée et, au fait, il ne serait donc pas nécessaire de nous occuper davantage de cette erreur. Peut-être cependant que, parmi les chimistes s'occupant de ce genre de recherches, il y en aura qui, dans les cas où à côté du lactose il y aura relativement peu de saccharose, préféreront faire usage de la table ancienne plutôt que de la nouvelle, et en tout cas, ceux qui ont déjà utilisé celle-là auront peut-être quelque intérêt

à apprendre de quel ordre de grandeur est l'erreur à laquelle elle a donné lieu. Nous allons donc y regarder de plus près.

Ainsi qu'il ressort du tableau 1, le traitement par acide a fait monter de 1.5 % le pouvoir réducteur du lactose. Or, comme la quantité de lactose dont il s'agit dans les déterminations où l'on aura employé la table V s'élève à 50 mgr, correspondant à 63<sup>mgr</sup>,3 de cuivre, il en résulte que l'accroissement de cuivre séparé dû au traitement par acide sera de 0<sup>mgr</sup>,9, ce qui, à son tour, aura pour effet que la teneur en sucre interverti se trouvera évaluée à un chiffre trop élevé de 0<sup>mgr</sup>,5, puisque 0<sup>mgr</sup>,9 de cuivre correspond à 0<sup>mgr</sup>,5 de sucre interverti. Toutefois, l'erreur ne sera aussi grande que là où la proportion de sucre interverti est très faible. Il est dans la donnée de la chose que plus est forte la quantité de sucre interverti présente, moins sera grande la quantité totale de cuivre réduit par les 50 mgr. de lactose, et moindre sera par conséquent l'erreur absolue de l'estimation du sucre interverti.

Afin de démontrer la justesse de cette considération, nous avons effectué une série d'essais d'après la Table V, mais avec du lactose »interverti d'après Nicol«, essais qui sont consignés au tableau 3.

Tableau 3.

50 mgr. lactose traité d'après Nicol.

Sucre interverti	Cu		Diff. T ÷ Table V
	Trouvé	D'après Table V	
0 .....	63.3	63.3	+ 0.0
0 .....	63.4	63.3	+ 0.1
24.70 .....	111.0	111.2	÷ 0.2
49.82 .....	157.3	158.1	÷ 0.8
74.87 .....	204.3	204.0	+ 0.3
125.02 .....	295.5	294.4	+ 1.1
149.87 .....	339.5	338.1	+ 1.4
149.96 .....	341.1	338.2	+ 2.9
175.12 .....	382.7	381.9	+ 0.8
200.68 .....	425.5	425.6	÷ 0.1
200.98 .....	424.4	426.2	÷ 1.8

Total 3.7

Il en résulte, on le voit, que l'augmentation totale de la quantité de cuivre dans les onze essais n'est que de 3<sup>mgr</sup>,7, alors que s'il s'en était séparé chaque fois 0<sup>mgr</sup>,9 de trop, cette augmentation aurait dû s'élever à 9<sup>mgr</sup>,9. En admettant que l'erreur diminue d'après une série arithmétique pour finir par se rapprocher de zéro à la fin du tableau, où la totalité du cuivre s'est séparée, le résultat sera que l'erreur calculée totale de ces 11 essais, qui sont assez uniformément répandus sur la Table V, se trouvera réduite à la moitié (4,95), donc aussi près de la valeur trouvée qu'on aurait pu s'y attendre. C'est dire que là où il y a eu beaucoup de sucre interverti, l'erreur est insignifiante, et même le chimiste qui se serait servi de notre Table V pour le dosage de faibles quantités de sucre interverti, n'aurait guère lieu de s'inquiéter.

Cependant, si à l'avenir on trouvait avantageux de recourir en certains cas à cette table, ce sera sans doute particulièrement à la partie qui se rapporte aux mélanges à relativement faible teneur en saccharose ou en sucre interverti, et rien n'empêchera de s'en servir, pourvu qu'en l'absence de sucre interverti l'on ait soin d'appliquer à la quantité de cuivre trouvée une correction de  $\div 0^{\text{mgr}},9$ , et qu'en présence de ce sucre on diminue de 0,1 par 25 mgr. de celui-ci la valeur numérique de cette correction. En tous cas, il ne semble pas qu'il y ait lieu d'établir une nouvelle table.

*En novembre 1925.*







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DES TRAVAUX

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# STUDIES ON PROTEINS.

BY

S. P. L. SØRENSEN.

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## IX. THE INFLUENCE OF SALT CONCENTRATION ON THE ACID-BINDING CAPACITY OF EGG-ALBUMIN.

BY

K. LINDERSTRØM-LANG AND ELLEN LUND.

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The greater part of the experiments dealt with in the following paper, viz, that part comprising the determination of  $f_H$ , the activity coefficient of the hydrogen ion in ammonium chloride solutions, and the determination of the ionisation of egg-albumin in ammonium chloride solutions, was carried out at the Carlsberg Laboratory in the years 1917—1918 by one of us, (Ellen Lund), with some assistance from Hr. cand. polyt. Sven Palitzsch. An extensive series of experiments for determination of  $f_H$  was, however, made later, in 1922, by the other of the present writers, in addition to which, a short series of experiments on the ionisation of egg-albumin in potassium chloride solutions was made in the autumn of 1925 by Dr. William A. Perlzweig (of the Johns Hopkins Hospital, Baltimore) during a period of study at the Carlsberg Laboratory. At the instigation of Prof. Sørensen, these researches have been collected, and, as regards the earlier experiments, new calculations made from the material, with due regard to modern views on the constitution of the solutions. We wish to take this opportunity of expressing our sincere thanks to Prof. Sørensen for his kind suggestion, and also to thank cand. polyt. Sven Palitzsch and Dr. Perlzweig for their valuable assistance.

### Introduction.

The capacity of proteins to combine with acids and bases is a phenomenon which has long been under consideration by scientists in this field of work. In the case of egg-albumin we may

mention works by J. Sjöquist<sup>1)</sup>, Bugarszky and Liebermann<sup>2)</sup>, J. Loeb<sup>3)</sup>, Hitchcock<sup>4)</sup>, W. Pauli<sup>5)</sup> and Cohn and Berggren<sup>6)</sup>. At the Carlsberg Laboratory, the capacity of egg-albumin to combine with acids in ammonium sulphate solutions has previously been investigated by S. P. L. Sørensen, with the collaboration of M. Høyrup, J. Hempel, and S. Palitzsch<sup>7)</sup>, and the experiments dealt with in the following pages are to be regarded as a natural continuation of the earlier work. They have been carried out in precisely the same way, with the exception of Dr. Perlzweig's measurements, which were effected by means of the quinhydrone electrode.

The theoretical side of the question has hitherto been dealt with only in rather rough outline, the egg-albumin being compared with a simple ampholyte such as for instance glycocoll, and certain peculiar qualities of the egg-albumin would thus escape attention. So also, it is only in certain of the more recent works by E. J. Cohn<sup>8)</sup>, Kinsuke Kondo<sup>9)</sup>, J. Frisch, W. Pauli and E. Valko<sup>10)</sup>, K. Linderström-Lang<sup>11)</sup>, K. Linderstrøm-Lang and S. Kodama<sup>12)</sup> and Northrop and Kunitz<sup>13)</sup> that protein chemistry has taken into consideration modern views as to the qualities of electrolytes. We shall have occasion to refer to these papers later on.

For the present, we will briefly note the principle in determination of the capacity to combine with acids:

To one litre of a solution of pure egg-albumin in pure water, containing  $c_N$  gram-equivalents of protein nitrogen, are added  $c_{BHS}$  equivalents of the salt BHS and  $Y$  equivalents of the

<sup>1)</sup> Skand. Arch. f. Physiol. **5**, 277. **6**, 255 (1895).

<sup>2)</sup> Arch. ges. Physiol. **72**, 51 (1898).

<sup>3)</sup> Journ. Gen. Physiol. **3**, 85 (1921).

<sup>4)</sup> *ibid.* **5**, 383 (1923).

<sup>5)</sup> Kolloidchemie d. Eiweisskörper. (Steinkopff) 1920.

<sup>6)</sup> Physiolog. Reviews **5**, No 3 (1925).

<sup>7)</sup> Compt.-rendus du Lab. Carlsberg **12**, 66 (1917).

<sup>8)</sup> *l. c.*

<sup>9)</sup> Compt.-rendus du Lab. Carlsberg **15**, No 8 (1924).

<sup>10)</sup> Biochem. Zeitschr. **164**, 401 (1925).

<sup>11)</sup> Compt.-rendus du Lab. Carlsberg **15**, No 7 (1924).

<sup>12)</sup> *ibid.* **16**, No 1 (1925).

<sup>13)</sup> Journ. of Gen. Physiol. **9**, 351 (1926).

acid HS or the base  $B^1$ ),  $Y$  being reckoned as positive in the first case, negative in the second.  $Y$  is assumed to be small in comparison with  $c_{BHS}$ , and if the egg-albumin, in addition to the hydrogen ion, also combines with the other ions  $BH^+$  and  $S^-$  then we likewise assume that the bound quantities of these are small in comparison with  $c_{BHS}$ . With a view to the application of the calculation to albumin solutions containing ammonium chloride, we will imagine the acid HS strong, while B is a weak base, though not too weak to render  $c_B$ , the concentration of the free base at the hydrogen ion activity given in the solution, insignificant in comparison with  $c_{BHS}$ .

Let  $\beta_H$  be the equivalent of hydrogen ions taken up or given off by the egg-albumin, positive in the former, negative in the latter case. We then have:

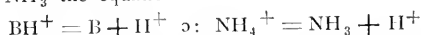
$$Y = c_H + \beta_H - c_B - c_{OH}. \quad (1)$$

Taking, for the »hydrolysis« of  $BH^+$  according to the formula

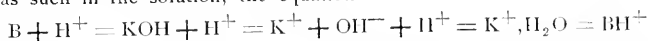
$$BH^+ = B + H^+ \quad (2)$$

<sup>1)</sup> We will here and in the following, in agreement with J. N. Brønsted (Rec. des Trav. Chim. d. Pays-Bas **42**, 718 (1923)) define an acid as being capable of giving off hydrogen ions, a base as capable of taking up hydrogen ions, and an ampholyte as capable of both. Any uncharged base we will therefore write as B, and any uncharged acid as HS. Any positive ion will then be  $BH^+$  and any negative  $S^-$ , where again  $BH^+$  is an acid,  $S^-$  a base insofar as B and HS can be formed in the solution.

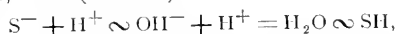
When  $B = NH_3$  the equation



is obvious for the equilibrium between the acid  $NH_4^+$  and the base  $NH_3$ , whereas for the base in an ordinary sense — KOH, which does not exist at all as such in the solution, the equation

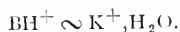


is more complex. According to Brønsted, it is in reality the OH ion which is the base, since (as above)



(and the negatively charged base  $OH^-$  is designated by  $S^-$ ) whereas the potassium ion, which is completely dissociated from the hydroxyl ion and has neither tendency to take up nor tendency to give off hydrogen ions, is indifferent in this connection. The potassium ion is also entirely eliminated from an equation such as (11).

There might perhaps be reason to formulate entirely new names, on the basis of Brønsted's formal principle, which is in many respects to be preferred, in order to avoid coming into conflict with the old ideas of acids and bases. There is however, hardly any occasion to do so here, as we shall hardly be misunderstood when we write:



the mass action equation

$$a_B \cdot a_H = k_B \cdot a_{BH^+} \quad (3)$$

where  $a_B$ ,  $a_H$  etc. denote the activity of the substance stated in the index, and with  $f_H$ ,  $f_B$  etc. similarly denoting the activity coefficients, we then, having regard to (1) and (3), to the equations

$$a_{BH^+} = f_{BH^+} \cdot c_{BH^+}, \quad a_B = f_B \cdot c_B \quad \dots \quad a_H = f_H \cdot c_H^1) \quad (4)$$

and to the equation

$$a_H \cdot a_{OH} = k_W \cdot a_{H_2O} \quad (k_W \text{ the diss. constant of water}) \quad (5)$$

obtain the following:

$$c_{OH} = \frac{k_W \cdot a_{H_2O}}{f_{OH} \cdot a_H} \quad (6)$$

$$c_B = c_{BH^+} \cdot \frac{k_B \cdot f_{BH^+}}{f_B \cdot a_H} \quad (7)$$

$$\beta_H = Y - \frac{a_H}{f_H} \left( 1 - \frac{k_W \cdot a_{H_2O} \cdot f_H}{f_{OH} \cdot a_H^2} \right) + c_{BH^+} \cdot \frac{k_B \cdot f_{BH^+}}{f_B \cdot a_H} \quad (8)$$

At the hydrogen ion activities and salt concentrations which we shall have to deal with in the following, the term

$$1 - \frac{k_W \cdot a_{H_2O} \cdot f_H}{f_{OH} \cdot a_H^2} \quad (9)$$

is very nearly 1.  $c_{BH^+}$  we can likewise, from the foregoing, take as equal to  $c_{BHS}$  the salt concentration, and we have then, from (8)

$$\beta_H = Y - \frac{a_H}{f_H} + c_{BHS} \cdot \frac{k_B \cdot f_{BH^+}}{f_B \cdot a_H}, \quad (10)$$

which holds good when B is a weak base.

In this equation, then, Y can have either plus or minus sign, according as there is added surplus of acid or of base.

Where B is not a weak base ( $BHS = KCl$  for instance)  $k_B$  can be taken as = 0, and we then obtain:

$$\beta_H = Y - \frac{a_H}{f_H} \quad (11)$$

Now in (10) or (11) we know Y, the quantity of acid or base added.  $a_H$  is measured electrometrically, and  $c_{BHS}$  is the salt concentration in the solution.

When therefore  $f_H$ ,  $f_{BH^+}$ ,  $f_B$  and  $k_B$  are also known,  $\beta_H$  can

<sup>1)</sup> For the activities and activity coefficients, their definition and thermodynamics, see e. g. Lewis & Randall: Thermodynamics and the Free Energy of Chemical Substances, Mc Graw-Hill Book Co. (1923).



be calculated. (When  $k_B$  is 0 it is only necessary to know  $f_H$ ). These values we will assume to be independent of  $c_N$  and thus determinable by the investigation of pure acid and salt solutions.

As the two last terms in (10) are only a correction of  $Y$  (we shall later, see p. 11, name this correction  $kH$ ) save in the vicinity of isoelectric reaction, where egg-albumin has its minimal charge and therefore presumably its activity influence is at its lowest, we shall hardly be very far wrong in this.

Of the following sections, the first, A, deals with the determination of  $f_H$ ,  $f_{BH^+}$ ,  $f_B$  and  $k_B$  for  $NH_4Cl$ . The second, B, gives the experimental results for acid binding of egg-albumin in ammonium chloride and potassium chloride solutions. The third, C, contains 1) a brief theoretical survey, 2) a summary of the experimental results 3) a determination of isoelectric reaction (or isoionic reaction, as we should prefer, for special reasons, to call the hydrogen ion activity at which  $\beta_H$  is 0, see p. 22) accompanied by a comparison with the value previously found at this laboratory in ammonium sulphate solutions, and 4) a calculation of the capacity to combine with acids from the ideas developed in a previous paper by one of the present writers, which permit a rough quantitative estimate of the variation of the ionisation with the salt concentration, attributing certain simple qualities to the egg-albumin.

### A. Determination of $f_H$ , with $f_{BH^+}$ , $f_B$ and $k_B$ for Ammonium Chloride Solutions.

#### 1) Determination of $f_H$ .

$f_H$  was measured in the following manner:

Stock solutions were prepared, of pure, recrystallised ammonium chloride, and of hydrochloric acid, the concentration being very exactly known. For each ammonium chloride concentration at which  $f_H$  was to be found, 4—8 solutions were prepared from the stock solution, to this concentration of ammonium chloride, and with varying hydrochloric acid concentrations, as a rule from 0.0005 to 0.002 n. The hydrogen ion activity was measured, the formula

$$p_{aH} = \frac{E - 0.3357}{0.0577 + 0.0002 (T - 18)^1} \quad (12)$$

<sup>1)</sup> S. P. L. Sørensen and K. Linderstrøm-Lang: Comptes-rendus du Lab. Carlsberg 15, No 6 (1924).

where  $E$  is the potential of the hydrogen electrode relative to the 0.1 *n* potassium chloride-calomel electrode, serving as the basis of the calculation. No account was taken of the liquid junction potential, which must be presumed to be very slight, as the velocity of migration for the ammonium, potassium and chlorine ions are very nearly alike even at higher concentration.

From these measurements,  $f_H$  was given graphically, with  $a_H$  as abscissa,  $c_{HCl}$  as ordinate. Since  $c_{HCl}$  was, in all our experiments, small in comparison with the ammonium chloride concentration, the curve thus produced will be a straight line, the direction tangent of which is  $f_H$ :

$$a_H = f_H \cdot c_{HCl}^1). \quad (13)$$

The hydrolysis of the ammonium chloride was of no importance in any of these experiments. (For details of the determination see S. P. L. Sorensen l. c.).

It should perhaps be pointed out that  $f_H$  is the so-called »apparent« activity coefficient<sup>2)</sup> for the hydrogen ion, and that  $a_H$  is the activity of the non-hydrated hydrogen ion.

The results are shown in Table I and Fig. 1.

In Fig. 1, the concentration of ammonium chloride is the ordinate, and  $f_H$  the abscisse. The salt concentration varies from 0.029 til 4.444 *n*.

The experiments with ammonium chloride marked I, II, III and IV were carried out in 1917—1918 with Hasselbalck's shaking electrode at ordinary indoor temperature, correction being made for the temperature by means of the term 0.0002 ( $T-18^\circ$ ) in (12) but not for barometric height. The experiments with ammonium chloride, M, were carried out in 1922 with the ordinary hydrogen electrode (hydrogen passed through) placed in a water thermostat at  $18^\circ \pm 0.02$ , correction being made for the difference in barometric height from 760 mm. The last-mentioned experiments must therefore be regarded as the most accurate, and the curve Fig. 1 is therefore drawn mainly on the basis of these. The last column in Table I gives the  $f_H$  values graphically found from the curve. The error here must be estimated at 1—2 %.

<sup>1)</sup> It should be pointed out that this graph method eliminates errors due to the fact that the ammonium chloride used does not contain  $NH_3$  and  $HCl$  in quite equivalent amounts (cf. S. P. L. Sorensen l. c.). This error is, however, as a rule slight compared with the experimental.

<sup>2)</sup> N. Bjerrum: Medd. f. Kungl. Vetensk. Nobelinst. 5, No 16 (1919).

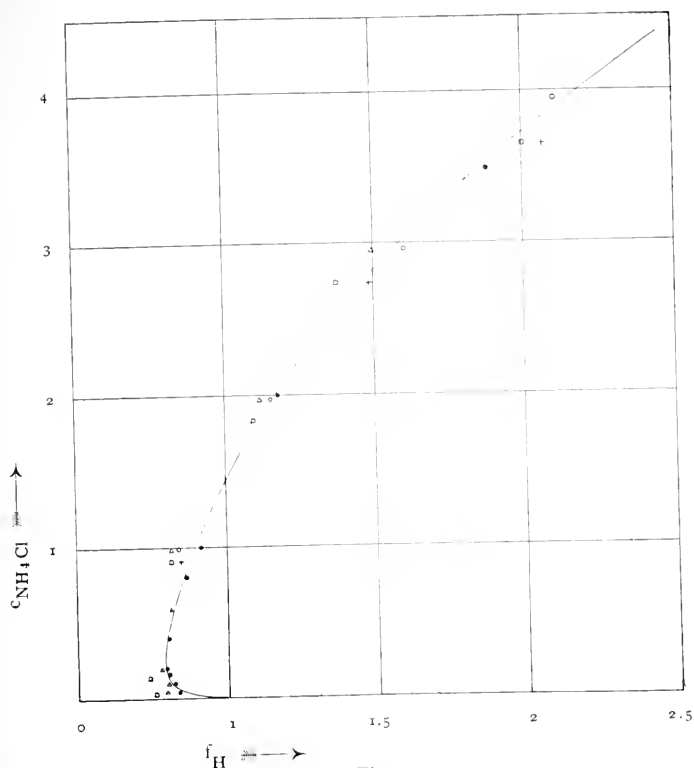


Fig. 1.

2) *Values of  $f_{\text{BH}^+}$ ,  $f_{\text{B}}$  and  $k_{\text{B}}$  for ammonium chloride and ammonia.*

At  $18^\circ$  in dilute aqueous solution, the dissociation constant of ammonium hydroxide is  $1.75 \cdot 10^{-5}$ , i. e.

$$c_{\text{NH}_4^+} = \frac{1.75 \cdot 10^{-5}}{k_{\text{W}}} \cdot c_{\text{NH}_3} \cdot c_{\text{H}} \quad (14)$$

as

$$c_{\text{H}} \cdot c_{\text{OH}} = k_{\text{W}} = 0.72 \cdot 10^{-14}$$

whence

$$c_{\text{NH}_3} = 0.41 \cdot 10^{-9} \cdot \frac{c_{\text{NH}_4^+}}{c_{\text{H}}} \quad (15)$$

$c_{\text{NH}_3}$  indicates the total concentration of ammonia (whether hydrated or not) in the solution. At higher salt concentration (concentration of ammonium chloride) the concentrations in (15) must be replaced by activities:

$$a_{\text{NH}_3} = 0.41 \cdot 10^{-9} \cdot \frac{a_{\text{NH}_4^+}}{a_{\text{H}}} \quad (16)$$

Table 1. Determinations of  $f_H$ .

$f_H$ direct						$f_H$ curve
NH <sub>4</sub> Cl, No:	I	II	III	IV	M	
Mrk. on curve Fig. 1	○	△	□	+	●	
<sup>c</sup> NH <sub>4</sub> Cl						
0.010						0.940
0.020						0.900
0.029			0.751			
0.040					0.830	
0.049		0.795				
0.050						0.848
0.098		0.800				
0.100					0.821	0.811
0.137			0.734			
0.161					0.801	
0.197		0.776				
0.200					0.795	0.792
0.401					0.804	
0.500						0.811
0.590		0.813				
0.802					0.865	
0.912			0.813	0.846		
0.985	0.839	0.815				
1.000					0.917	0.894
1.824			1.095	1.094		
1.970	1.152	1.091				
2.000					1.179	1.167
2.738			1.375	1.489		
2.956	1.602	1.497				
3.000						1.583
3.490					1.880	
3.650			2.004	2.068		
3.950	2.106					
4.000						2.175
4.444	2.345					

or

$$c_{\text{NH}_3} = 0.41 \cdot 10^{-9} \cdot \frac{f_{\text{NH}_4^+}}{f_{\text{NH}_3}} \cdot \frac{c_{\text{NH}_4\text{Cl}}}{a_{\text{H}}} \quad (17)$$

the hydrogen ion activity being assumed to be so high that the concentration of ammonium ions is practically equal to that of the ammonium chloride. On comparing with (7) we see that  $k_B = 0.41 \cdot 10^{-9}$ , when  $c_{\text{BHS}} = c_{\text{NH}_4\text{Cl}} = c_{\text{BH}^+} = c_{\text{NH}_4^+}$ ,  $f_{\text{BH}^+} = f_{\text{NH}_4^+}$  and  $f_B = f_{\text{NH}_3}$ .

$f_{\text{NH}_4^+}$  we will take as equal to the activity coefficient of the potassium ion given in Table 2 after H. Harned<sup>1)</sup> at the salt concentrations we shall have to deal with in the following:

Table 2.

$c_{\text{NH}_4\text{Cl}}$	$f_{\text{NH}_4^+} = f_{\text{K}^+}$
0.05	0.80
0.2	0.70
1.0	0.58
3.0	0.56

The error here involved is doubtless very slight, owing to the great similarity between the ammonium ion and the potassium ion. Moreover, in the determination of  $\beta_{\text{H}}$  after (10),  $f_{\text{NH}_4^+}$  enters in as a correction term, slight in the first place as compared with the quantity of acid added (Y) and further, but inexactly determined in itself, since we have to disregard the activity influence of the egg-albumin. We have therefore considered it unnecessary to devote more attention to this point.

$f_{\text{NH}_3}$  can be found from the ammonia tension of solutions of ammonia and ammonium chloride<sup>2)3)4)</sup>. This vapour tension is now practically speaking independent of the ammonium chloride concentration, so that we can take  $f_{\text{NH}_3}$  as = 1 at all concentrations. We thus obtain, from (17)

$$c_{\text{NH}_3} = 0.41 \cdot 10^{-9} \cdot f_{\text{NH}_4^+} \cdot \frac{c_{\text{NH}_4\text{Cl}}}{a_{\text{H}}} \quad (18)$$

As regards the activity coefficient of the hydrogen ion in the potassium chloride-ammonium chloride solutions later used

<sup>1)</sup> Journ. Amer. Chem. Soc. **42**, 1808 (1920).

<sup>2)</sup> Gaus: Zeitschr. f. anorg. Chem **25**, 236 (1900).

<sup>3)</sup> Abegg and Riesenfeld: Zeitschr. f. physik. Chem, **40**, 84 (1902).

<sup>4)</sup> Riesenfeld: Zeitschr. f. physik. Chem. **45**, 461 (1903).

(p. 11) ( $c = 0.006 - 0.0206$ ) we have employed the same values as found in the previous section for pure ammonium chloride solutions.

## B. Experiments with the Acid- and Base-Binding Capacity of Egg-Albumin.

### 1) *Egg-Albumin. Ammonium Chloride and Hydrochloric Acid.*

#### a) Method.

The experiments were carried out as follows:

A portion of egg-albumin 6 times recrystallised from ammonium sulphate solution was dissolved in water and dialysed with the addition of small quantities of ammonia until all sulphate ions had disappeared. After dialysis, the quantity of ammonia was determined, and the quantity of protein nitrogen in the dialysed solution ascertained (see S. P. L. Sørensen »Studies on Proteins«<sup>1)</sup>). The amount of hydrochloric acid required to neutralise the ammonia was then added. The solution thus prepared was used as a stock solution. Stock solutions of ammonium chloride and hydrochloric acid were also prepared, and in preparing a desired egg-albumin-ammonium-chloride-hydrochloric-acid solution, the fixed quantities of the three stock solutions were weighed in a 50 cc measuring flask which was then filled up to 50 cc. The hydrogen ion activity was measured by Hasselbalck's shaking electrode in the same way as with measurement of  $f_H$  (p. 6) the liquid junction potential being here disregarded. As a rule, the determinations were made in triplicate, and all results showing more than 0.02  $\text{p}a_H$  discrepancy between the three have been discarded.

#### b) The concentrations and egg-albumin samples used.

Two different egg-albumin samples were employed, viz. D. Æ. 14 and D. Æ. 17 B, both treated in the same way.  $c_N$  was varied from 0.05 to 0.39,  $Y$  from  $-30 \cdot 10^{-4}$  to  $140 \cdot 10^{-4}$  and  $c_{\text{NH}_4\text{Cl}}$  from 0.05 n to 3 n.

#### c) Terms employed.

$c_{\text{NH}_4\text{Cl}}$ : gram-equivalents of  $\text{NH}_4\text{Cl}$  per litre protein solution.

$Y$ : gram-equivalents of acid or base per litre protein solution.

<sup>1)</sup> Compt.-rend. du Lab. Carlsberg **12** p. 24, 48, 120. (1917).

$c_N$ : gram-equivalents protein nitrogen per litre protein solution.

$kH$ :  $-a_H/f_H + c_{NH_4Cl} \cdot 0.41 \cdot 10^{-9} \cdot f_{NH_4^+}/a_H$  the correction noted on p. 5 (see (10) and (18)).

$\beta_H$ :  $Y + kH$ , the equivalents of hydrogen ions combined with the egg-albumin per litre.

$\bar{H}$ :  $\frac{\beta_H}{c_N}$  no. of hydrogen ions combined (or given off) per gram equivalents protein nitrogen.

$\bar{h}$ :  $\frac{\beta_H}{c_A}$  no. of combined hydrogen ions pr. gram-molecule egg-albumin. (Molecular weight of egg-albumin 35000)  $c_A$  is the molar concentration of egg-albumin; an egg-albumin molecule contains 357 nitrogen atoms,  $c_N = 357 \cdot c_A$  (see p. 43).

d) Observations on the surplus of HCl of  $NH_3$  in the ammonium chloride.

If the ammonium chloride used for the experiments does not contain equivalent quantities of acid and base, this must be taken into consideration in determination of  $Y$ , which cannot then be taken as equal to the added quantity of acid or base. The error involved by disregarding this possibility is however, as mentioned (p. 6), slight in comparison with the experimental error in determination of hydrogen ion activity. Only in a single instance have we, for reasons which will be mentioned in the section dealing with isoionic reaction of egg-albumin (p. 23 and 39) considered it necessary to re-calculate  $Y$  from the assumption that the ammonium chloride contained a surplus of base. This was in the experiment with D. Æ. 14, Tables 5 and 5 a, where the former table contains the uncorrected values, the latter the corrected figures (see p. 14 and 15).

#### e) Results.

The results are given in Tables 3 to 9, p. 12—19.

#### 2) *Egg-Albumin, Potassium Chloride and Hydrochloric Acid.*

The experiments were made in a manner similar to that previously employed, save that the hydrogen ion activity was determined at  $18^\circ \pm 0.02$  by means of the quinhydrone electrode<sup>1)</sup>

<sup>1)</sup> Einar Biilmann: Universitetets Festskrift 1920. Ann. d. Chimie **15**, 109 (1921).

Table 3.

D. A.E. 14 in $\text{NH}_4\text{Cl}$ . $c_{\text{NH}_4\text{Cl}} = 0.0500$ ; $f_{\text{H}} = 0.85$						
$c_{\text{N}}$	$Y \cdot 10^4$	$\text{p}a_{\text{H}}$	$\text{kH} \cdot 10^4$	$\beta_{\text{H}} \cdot 10^4$	$\bar{H} \cdot 10^3$	$\bar{h}$
0.3896	80.59	4.131	- 0.87	79.72	20.46	7.30
0.3927	68.23	4.240	- 0.68	67.55	17.20	6.14
0.3899	56.78	4.343	- 0.54	56.24	14.42	5.15
0.3926	41.14	4.471	- 0.40	40.74	10.38	3.70
0.3901	27.94	4.607	- 0.29	27.65	7.09	2.53
0.3898	11.84	4.776	- 0.20	11.64	2.99	1.07
0.3930	0.00	4.873	- 0.16	- 0.16	- 0.04	- 0.01
0.3902	- 27.98	5.208	- 0.07	- 28.05	- 7.19	- 2.57
0.1299	27.58	4.129	- 0.88	26.70	20.55	7.33
0.1292	23.76	4.221	- 0.71	23.05	17.84	6.37
0.1299	19.82	4.318	- 0.57	19.25	14.82	5.29
0.1292	15.79	4.437	- 0.43	15.36	11.89	4.24
0.1303	11.82	4.524	- 0.35	11.47	8.80	3.14
0.1291	7.92	4.650	- 0.26	7.66	5.93	2.12
0.1302	3.95	4.761	- 0.20	3.75	2.88	1.03
0.1292	0.00	4.893	- 0.15	- 0.15	- 0.11	- 0.04
0.1291	- 6.38	5.101	- 0.09	- 6.47	- 5.01	- 1.79
0.0525	11.45	4.134	- 0.87	10.58	20.15	7.19
0.0523	10.86	4.180	- 0.78	10.08	19.27	6.88
0.0520	9.08	4.262	- 0.65	8.43	16.21	5.79
0.0525	7.90	4.323	- 0.56	7.34	13.98	4.99
0.0509	5.94	4.406	- 0.46	5.48	10.77	3.84
0.0525	3.97	4.577	- 0.31	3.66	6.97	2.49
0.0513	1.98	4.698	- 0.24	1.74	3.39	1.21
0.0527	0.00	4.879	- 0.16	- 0.16	- 0.30	- 0.11
0.0525	- 3.20	5.170	- 0.08	- 3.28	- 6.25	- 2.23

and correction made for the salt- and protein error<sup>1)</sup>.  $\text{p}a_{\text{H}}$  was calculated from the formula:

$$\text{p}a_{\text{H}} = \frac{0.3691 - E_{\text{kin}}}{0.0577} + Q \quad (19)$$

where  $E_{\text{kin}}$  is the potential difference between the quinhydrone electrode and the 0.1 N potassium-chloride-calomel electrode. The

<sup>1)</sup> K. Linderström-Lang: Compt.-rendus Lab. Carlsberg **16**, No 3 (1925).



Table 4.

D. Æ. 14 in  $\text{NH}_4\text{Cl}$ .  $c_{\text{NH}_4\text{Cl}} = 0.200$ ;  $f_{\text{H}} = 0.79$ .

$c_{\text{N}}$	$Y \cdot 10^4$	$p_{\text{aH}}$	$k_{\text{H}} \cdot 10^4$	$\beta_{\text{H}} \cdot 10^4$	$H \cdot 10^3$	$h$
0.3900	100.48	4.050	-1.12	99.36	25.48	9.09
0.3910	96.40	4.139	-0.91	95.49	24.42	8.72
0.3929	92.98	4.173	-0.84	92.14	23.45	8.37
0.3943	80.69	4.241	-0.72	79.97	20.28	7.24
0.3930	70.17	4.325	-0.59	69.58	17.70	6.32
0.3930	55.78	4.436	-0.44	55.34	14.08	5.03
0.3943	39.86	4.558	-0.33	39.53	10.03	3.58
0.3930	20.09	4.711	-0.22	19.87	5.06	1.81
0.3945	0.00	4.890	-0.12	-0.12	-0.03	-0.01
0.3931	-20.01	5.094	-0.04	-20.05	-5.10	-1.82
<hr/>						
0.1316	35.75	4.099	-1.00	34.75	26.41	9.43
0.1306	29.76	4.210	-0.77	28.99	22.19	7.92
0.1315	23.78	4.347	-0.56	23.22	17.66	6.30
0.1316	15.90	4.524	-0.36	15.54	11.81	4.21
0.1315	7.95	4.701	-0.22	7.73	5.88	2.10
0.1316	3.93	4.808	-0.17	3.76	2.86	1.02
0.1316	0.00	4.919	-0.10	-0.10	-0.08	-0.03
0.1316	-6.40	5.107	-0.03	-6.43	-4.89	-1.75
<hr/>						
0.0525	15.75	4.076	-1.05	14.70	28.00	9.99
0.0529	13.79	4.171	-0.84	12.95	24.48	8.74
0.0528	11.84	4.256	-0.70	11.14	21.11	7.54
0.0522	9.92	4.351	-0.55	9.37	17.95	6.41
0.0530	7.90	4.454	-0.43	7.47	14.09	5.03
0.0529	3.95	4.669	-0.24	3.71	7.01	2.50
0.0522	1.99	4.795	-0.17	1.82	3.49	1.25
0.0522	0.00	4.923	-0.10	-0.10	-0.20	-0.07
0.0522	-3.16	5.184	-0.00	-3.16	-6.05	-2.16

liquid junction potential was disregarded.  $Q$  is the correction for egg-albumin- and salt error.

Only 1 egg-albumin sample was used, D. Æ. 23, which was treated in the same way as before. As these determinations were solely designed to ascertain the variation of  $\bar{H}$  with the acid concentration and salt concentration, not to finding its absolute value, the ammonia content was but roughly estimated. In the

Table 5.

D. AE. 14 in  $\text{NH}_4\text{Cl}$ .  $c_{\text{NH}_4\text{Cl}} = 0.990$ ;  $f_H = 0.89$ .

$c_N$	$Y \cdot 10^4$	$\rho_{\text{aH}}$	$kH \cdot 10^4$	$\beta_H \cdot 10^4$	$\bar{H} \cdot 10^3$	$\bar{h}$
0.3902	116.17	4.140	-0.78	115.39	29.57	10.55
0.3909	115.97	4.158	-0.75	115.22	29.48	10.52
0.3906	105.30	4.223	-0.63	104.67	26.80	9.56
0.3911	95.32	4.284	-0.56	94.76	24.23	8.65
0.3905	84.31	4.353	-0.45	83.86	21.48	7.67
0.3909	68.51	4.445	-0.34	68.17	17.44	6.22
0.3909	51.40	4.562	-0.23	51.17	13.09	4.67
0.3907	39.65	4.635	-0.16	39.49	10.11	3.61
0.3905	31.31	4.699	-0.10	31.21	7.99	2.85
0.3910	16.03	4.803	-0.03	16.00	4.09	1.46
0.3910	0.00	4.921	+0.06	0.06	0.02	0.01
0.3906	-33.94	5.265	+0.36	-33.58	-8.60	-3.07
0.1300	41.62	4.111	-0.84	40.78	31.37	11.20
0.1322	39.81	4.159	-0.75	39.06	29.55	10.55
0.1301	33.78	4.249	-0.59	33.19	25.51	9.10
0.1321	27.74	4.371	-0.43	27.31	20.67	7.38
0.1322	19.99	4.517	-0.26	19.73	14.92	5.32
0.1300	13.89	4.607	-0.19	13.70	10.54	3.76
0.1301	11.91	4.661	-0.14	11.77	9.05	3.23
0.1321	8.03	4.765	-0.05	7.98	6.04	2.16
0.1321	4.01	4.843	-0.00	4.01	3.04	1.08
0.1322	0.00	4.948	+0.07	0.07	0.05	0.02
0.1300	-5.27	5.073	+0.17	-5.10	-3.92	-1.40
0.0525	19.87	4.010	-1.10	18.77	35.75	12.75
0.0510	17.82	4.081	-0.93	16.89	33.12	11.80
0.0529	15.79	4.188	-0.69	15.10	28.54	10.19
0.0511	13.80	4.251	-0.59	13.21	25.85	9.23
0.0521	11.90	4.341	-0.46	11.44	21.96	7.84
0.0520	7.96	4.503	-0.28	7.68	14.77	5.27
0.0522	3.96	4.709	-0.10	3.86	7.39	2.64
0.0511	1.99	4.827	-0.01	1.98	3.87	1.38
0.0523	0.00	4.946	+0.07	0.07	0.13	0.05
0.0506	-2.39	5.081	+0.19	-2.20	-4.35	-1.55
0.0515	-3.28	5.135	+0.23	-3.05	-5.92	-2.11

Table 5 a.

D.  $\text{Æ. 14}$  in  $\text{NH}_4\text{Cl}$ .  $c_{\text{NH}_4\text{Cl}} = 0.990$ ;  $f_{\text{H}} = 0.89$ .

Corrected for surplus base in the ammonium chloride solution.

 $1.00.10^{-4}$  equivalents of base per equivalent of ammoniumchloride.

$c_{\text{N}}$	$Y \cdot 10^4$ corr.	$\text{p}a_{\text{H}}$	$\rho_{\text{H}} \cdot 10^4$	$\overline{\text{H}} \cdot 10^3$	$h$
0.39	115.17	4.140	114.39	29.31	10.46
—	114.97	4.158	114.22	29.22	10.43
—	104.30	4.223	103.67	26.54	9.47
—	94.32	4.284	93.76	23.97	8.56
—	83.31	4.353	82.86	21.22	7.58
—	67.51	4.445	67.17	17.18	6.13
—	50.40	4.562	50.17	12.83	4.58
—	38.65	4.635	38.49	9.85	3.52
—	30.31	4.699	30.21	7.73	2.76
—	15.03	4.803	15.00	3.83	1.37
—	— 1.00	4.921	— 0.94	— 0.24	— 0.08
—	— 34.94	5.265	— 34.58	— 8.86	— 3.16
0.13	40.62	4.111	39.78	30.61	10.92
—	38.81	4.159	38.06	28.79	10.28
—	32.78	4.249	32.19	24.75	8.83
—	26.74	4.371	26.31	19.91	7.11
—	18.99	4.517	18.73	14.16	5.05
—	12.89	4.607	12.70	9.78	3.49
—	10.91	4.661	10.77	8.29	2.96
—	7.03	4.765	6.98	5.28	1.89
—	3.01	4.843	3.01	2.28	0.81
—	— 1.00	4.948	— 0.93	— 0.71	— 0.29
—	— 6.27	5.073	— 6.10	— 4.68	— 1.67
0.05	18.87	4.010	17.77	33.85	12.08
—	16.82	4.081	15.89	31.16	11.12
—	14.79	4.188	14.10	26.65	9.51
—	12.80	4.251	12.21	23.89	8.53
—	10.90	4.341	10.44	20.04	7.15
—	6.96	4.503	6.68	12.85	4.59
—	2.96	4.709	2.86	5.48	1.96
—	0.99	4.827	0.98	1.92	0.69
—	— 1.00	4.946	— 0.93	— 1.78	— 0.64
—	— 3.39	5.081	— 3.20	— 6.32	— 2.26
—	— 4.28	5.135	— 4.05	— 7.86	— 2.81

Table 6.

D.E. 14 in  $\text{NH}_4\text{Cl}$ .  $c_{\text{NH}_4\text{Cl}} = 2.96$ ;  $f_H = 1.58$ .

$c_N$	$Y \cdot 10^4$	$p_{aH}$	$kH \cdot 10^4$	$\beta_H \cdot 10^4$	$\bar{H} \cdot 10^3$	$\bar{h}$
0.3887	135.74	4.066	-0.43	135.41	34.81	12.42
0.3893	119.49	4.165	-0.33	119.16	30.61	10.92
0.3885	101.00	4.276	-0.21	100.79	25.94	9.26
0.3891	80.37	4.386	-0.06	80.31	20.64	7.37
0.3890	59.82	4.511	+0.02	59.84	15.38	5.49
0.3891	40.68	4.628	+0.13	40.81	10.49	3.74
0.3885	19.69	4.771	+0.27	19.96	5.14	1.83
0.3891	0.00	4.909	+0.45	0.45	0.12	0.04
0.3858	-27.77	5.149	+0.89	-26.88	-6.97	-2.49
0.1313	42.30	4.114	-0.40	41.90	31.91	11.39
0.1287	39.63	4.159	-0.35	39.28	30.52	10.89
0.1312	35.53	4.247	-0.23	35.30	26.91	9.60
0.1287	33.73	4.257	-0.23	33.50	26.03	9.29
0.1287	27.74	4.385	-0.10	27.64	21.48	7.67
0.1303	19.89	4.529	+0.05	19.94	15.28	5.45
0.1313	14.21	4.617	+0.12	14.33	10.91	3.89
0.1304	7.99	4.761	+0.26	8.25	6.33	2.26
0.1287	3.98	4.818	+0.37	4.35	3.38	1.21
0.1305	0.00	4.913	+0.44	0.44	0.34	0.12
0.1312	-5.96	5.052	+0.68	-5.28	-4.02	-1.43
0.0527	17.84	4.129	-0.38	17.46	33.13	11.82
0.0527	15.86	4.209	-0.30	15.56	29.53	10.54
0.0524	11.78	4.369	-0.11	11.67	22.27	7.95
0.0525	8.92	4.486	0.00	8.92	16.99	6.06
0.0527	7.97	4.529	+0.05	8.02	15.22	5.43
0.0527	4.00	4.691	+0.19	4.19	7.95	2.84
0.0525	2.08	4.787	+0.31	2.39	4.55	1.62
0.0510	0.83	4.854	+0.38	1.21	2.37	0.85
0.0525	0.00	4.886	+0.42	0.42	0.80	0.29
0.0525	-1.58	4.994	+0.60	-0.98	-1.87	-0.67
0.0504	-3.20	5.086	+0.75	-2.45	-4.86	-1.73

solutions of egg-albumin, potassium chloride and hydrochloric acid made ready for measurements, the concentration of ammonium chloride was only 0.006 N, and Y was so corrected as to

Table 7.

D.  $\text{Æ. 17 B}$  in  $\text{NH}_4\text{Cl}$ .  $c_{\text{NH}_4\text{Cl}} = 0.200$ ;  $f_{\text{H}} = 0.79$ .

$c_{\text{N}}$	$Y \cdot 10^4$	$p_{\text{aH}}$	$k_{\text{H}} \cdot 10^4$	$\beta_{\text{H}} \cdot 10^4$	$\bar{H} \cdot 10^3$	$\bar{h}$
0.3829	99.88	4.084	-1.03	98.85	25.82	9.22
0.3829	88.14	4.162	-0.86	87.28	22.79	8.13
0.3830	72.49	4.281	-0.65	71.84	18.76	6.70
0.3807	61.22	4.368	-0.53	60.69	15.94	5.69
0.3804	39.98	4.528	-0.36	39.62	10.42	3.72
0.3803	19.93	4.694	-0.23	19.70	5.18	1.85
0.3804	0.00	4.870	-0.13	- 0.13	- 0.03	- 0.01
0.3811	- 20.10	5.078	-0.04	- 20.14	- 5.28	- 1.85
.						
0.1291	31.98	4.132	-0.92	31.06	24.06	8.59
0.1268	27.86	4.209	-0.77	27.09	21.36	7.62
0.1289	24.02	4.316	-0.60	23.42	18.17	6.48
0.1289	15.99	4.468	-0.41	15.58	12.09	4.31
0.1267	11.88	4.567	-0.32	11.56	9.12	3.25
0.1288	8.12	4.661	-0.25	7.87	6.11	2.18
0.1290	4.06	4.761	-0.19	3.87	3.00	1.07
0.1295	0.00	4.879	-0.13	- 0.13	- 0.10	- 0.04
0.1269	- 6.41	5.051	-0.04	- 6.45	- 5.08	- 1.81
0.0510	14.01	4.111	-0.97	13.04	25.57	9.12
0.0509	12.02	4.204	-0.78	11.24	22.08	7.88
0.0511	10.00	4.302	-0.62	9.38	18.36	6.55
0.0506	8.12	4.414	-0.47	7.65	15.12	5.40
0.0507	4.07	4.633	-0.26	3.81	7.51	2.68
0.0510	1.93	4.749	-0.20	1.73	3.39	1.21
0.0508	0.00	4.902	-0.12	- 0.12	- 0.21	- 0.07
0.0508	- 3.23	5.141	-0.01	- 3.24	- 6.38	- 2.28

make the point of intersection between the ionisation curves answering to different potassium chloride concentrations ( $\bar{H}$ ,  $p_{\text{aH}}$  curves see Fig. 5) coincide with the corresponding point of intersection for the ammonium chloride experiments.

$c_{\text{N}}$  was kept constant at 0.210, while the potassium chloride concentration varied from 0 to 0.2. The total salt concentration thus varied from 0.006 to 0.206.  $\beta_{\text{H}}$  was determined after (11). The results are given in Table 10, p. 20.

Table 8.

D. Æ. 17 B in  $\text{NH}_4\text{Cl}$ .  $c_{\text{NH}_4\text{Cl}} = 0.990$ ;  $f_{\text{H}} = 0.89$ .

$c_{\text{N}}$	$Y \cdot 10^4$	$\text{pa}_{\text{H}}$	$\text{kH} \cdot 10^4$	$\beta_{\text{H}} \cdot 10^4$	$\bar{H} \cdot 10^3$	$\bar{h}$
0.3848	118.77	4.096	-0.87	117.90	30.64	10.94
0.3869	101.24	4.199	-0.67	100.57	26.01	9.28
0.3850	79.03	4.341	-0.47	78.56	20.41	7.28
0.3868	60.50	4.457	-0.33	60.17	15.56	5.55
0.3868	39.43	4.597	-0.19	39.24	10.15	3.62
0.3868	19.21	4.753	-0.07	19.14	4.95	1.77
0.3849	0.00	4.896	+0.03	0.03	0.01	0.00
0.3876	-20.00	5.075	+0.18	-19.82	-5.11	-1.82
0.1267	40.05	4.090	-0.88	39.17	30.91	11.03
0.1272	33.94	4.196	-0.68	33.26	26.15	9.33
0.1271	27.94	4.305	-0.51	27.43	21.59	7.71
0.1278	20.12	4.480	-0.30	19.82	15.52	5.54
0.1270	14.09	4.568	-0.22	13.87	10.93	3.90
0.1299	7.96	4.716	-0.09	7.87	6.07	2.17
0.1297	3.94	4.803	-0.03	3.91	3.02	1.08
0.1289	0.00	4.880	+0.02	0.02	0.02	0.01
0.1301	-5.94	5.044	+0.15	-5.79	-4.44	-1.58
0.0517	16.98	4.098	-0.87	16.11	31.16	11.12
0.0516	14.40	4.202	-0.67	13.73	26.61	9.50
0.0517	11.78	4.310	-0.50	11.28	21.82	7.79
0.0516	7.88	4.491	-0.28	7.60	14.73	5.26
0.0514	3.94	4.678	-0.13	3.81	7.41	2.64
0.0515	0.00	4.888	+0.03	0.03	0.06	0.02
0.0511	-3.22	5.077	+0.19	-3.03	-5.93	-2.12

3) *Egg-albumin, Ammonium Sulphate and Sulphuric Acid.*

We shall later on (p. 37 and 49—53) have occasion to compare our experiments with the previous ones by S. P. L. Sørensen and his collaborators. We therefore give in Table 11 some of the values found for egg-albumin-ammonium-sulphate-sulphuric-acid solutions (see »Studies on Proteins« p. 126—127);  $\text{pa}_{\text{H}}$  is calculated from the  $\text{p}_{\text{H}}$  values by means of the formula

$$\text{pa}_{\text{H}} = \text{p}_{\text{H}} + 0.041 \quad (20)$$

(cf. S. P. L. Sørensen and K. Linderstrøm-Lang l. c.). The

**Table 9.**D. Æ. 17 B in  $\text{NH}_4\text{Cl}$ .  $c_{\text{NH}_4\text{Cl}} = 2.96$ ;  $f_{\text{H}} = 1.58$ .

$c_{\text{N}}$	$Y \cdot 10^4$	$\text{pa}_{\text{H}}$	$\text{kH} \cdot 10^4$	$\beta_{\text{H}} \cdot 10^4$	$\text{H} \cdot 10^3$	$\bar{h}$
0.3812	120.00	4.147	-0.36	119.64	31.39	11.20
0.3807	99.88	4.260	-0.23	99.65	26.18	9.34
0.3807	80.29	4.373	-0.10	80.19	21.06	7.52
0.3868	60.66	4.483	-0.01	60.65	15.68	5.60
0.3869	40.78	4.587	+0.10	40.88	10.57	3.77
0.3865	20.03	4.747	+0.26	20.29	5.25	1.87
0.3865	0.00	4.897	+0.44	0.44	0.11	0.04
0.3807	-26.06	5.118	+0.82	-25.24	-6.63	-2.37
0.1266	40.12	4.146	-0.36	39.76	31.41	11.21
0.1277	34.11	4.255	-0.23	33.88	26.53	9.47
0.1281	28.75	4.345	-0.13	28.62	22.34	7.97
0.1280	20.30	4.492	0	20.30	15.86	5.66
0.1278	14.15	4.597	+0.10	14.25	11.15	3.98
0.1284	8.13	4.712	+0.22	8.35	6.50	2.32
0.1282	4.04	4.794	+0.31	4.35	3.38	1.21
0.1287	0.00	4.900	+0.44	0.44	0.34	0.12
0.1266	-6.13	5.035	+0.66	-5.47	-4.32	-1.54
0.0506	17.40	4.133	-0.36	17.04	33.68	12.02
0.0506	14.59	4.240	-0.25	14.34	28.34	10.11
0.0514	12.12	4.338	-0.14	11.98	23.31	8.32
0.0514	8.07	4.509	+0.02	8.09	15.74	5.62
0.0513	3.96	4.689	+0.19	4.15	8.09	2.89
0.0506	2.03	4.746	+0.26	2.29	4.53	1.62
0.0514	0.00	4.881	+0.42	0.42	0.82	0.29
0.0507	-3.23	5.046	+0.67	-2.56	-5.05	-1.80

meaning of  $\mu$  will be further discussed later on,  $c$  is the concentration of ammonium sulphate.

### C. Theoretical Treatment of the Experimental Material.

#### 1) Brief Theoretical Survey.

##### a) Mean valency and specific hydrogen ionisation.

In a solution containing an ampholyte, e. g. egg-albumin, at the concentration  $c_{\text{A}}$  (the total number of particles into which the

Table 10.

D. Æ. 23 in  $\text{NH}_4\text{Cl} + \text{KCl}$ .

$c_N$	$Y \cdot 10^4$	$p_{\text{aH}}$	$c_H \cdot 10^4$	$\beta_H \cdot 10^4$	$\bar{H} \cdot 10^3$	$\bar{h}$
$c = 0.006, f_H = 0.95.$						
0.210	48.2	3.853	1.46	46.74	22.26	7.94
—	35.7	4.093	0.85	34.85	16.60	5.92
—	23.2	4.333	0.49	22.71	10.81	3.86
—	10.7	4.618	0.25	10.45	4.98	1.78
—	-1.8	4.951	0.12	-1.92	-0.91	-0.32
$c = 0.056, f_H = 0.85.$						
0.210	47.5	4.095	0.94	46.56	22.17	7.91
—	35.0	4.280	0.62	34.38	16.37	5.84
—	22.5	4.479	0.39	22.11	10.53	3.76
—	10.0	4.701	0.24	9.76	4.65	1.66
—	-2.5	4.953	0.13	-2.63	-1.25	-0.45
$c = 0.106, f_H = 0.81.$						
0.210	47.0	4.158	0.86	46.14	21.97	7.84
—	34.5	4.331	0.58	33.92	16.15	5.76
—	22.0	4.523	0.37	21.63	10.30	3.68
—	9.5	4.725	0.23	9.27	4.41	1.57
—	-3.0	4.957	0.14	-3.14	-1.49	-0.53
$c = 0.206, f_H = 0.79.$						
0.210	47.0	4.229	0.75	46.25	22.02	7.86
—	34.5	4.384	0.56	33.94	16.16	5.76
—	22.0	4.563	0.34	21.66	10.31	3.68
—	9.5	4.763	0.22	9.28	4.41	1.57
—	-3.0	4.949	0.14	-3.14	-1.49	-0.53

ampholyte enters) and the ions  $\text{BH}^+$ ,  $\text{S}^-$  and  $\text{H}^+$ , let the quantities of these ions combined or given off by the egg-albumin be  $\beta_{\text{BH}^+}$ ,  $\beta_{\text{S}^-}$  and  $\beta_{\text{H}^+}$ . As the ampholyte is assumed to be »in itself« neutral, not containing the ions  $\text{BH}^+$  and  $\text{S}^-$ , it follows that  $\beta_{\text{BH}^+}$  and  $\beta_{\text{S}^-}$  will be positive, answering to the number of ions taken up, whereas  $\beta_{\text{H}^+}$  can be either positive or negative. The mean valency  $\bar{r}$  of the ampholyte is then defined by the equation:

$$\bar{r} = \frac{\beta_{\text{H}^+} + \beta_{\text{BH}^+} - \beta_{\text{S}^-}}{c_A} \quad (21)$$



Table 11.

Egg-albumin in  $(\text{NH}_4)_2\text{SO}_4$ .

$c_N$	$p_{aH}$	$\bar{H} \cdot 10^3$	$\bar{h}$
$c = 0.059. \mu = 0.089.$			
0.317	4.034	26.0	9.28
—	4.100	23.4	8.36
—	4.190	20.6	7.35
—	4.281	17.5	6.25
—	4.387	13.9	4.97
—	4.543	9.3	3.32
—	4.722	3.5	1.25
—	4.858	0.0	0.00
0.105	4.047	25.3	9.03
—	4.109	22.9	8.17
—	4.191	20.5	7.32
—	4.287	17.5	6.25
—	4.379	13.8	4.93
—	4.515	9.4	3.35
—	4.710	3.5	1.25
—	4.835	0.0	0.00
$c = 0.354. \mu = 0.531.$			
0.320	4.146	25.2	8.90
—	4.192	23.3	8.32
—	4.251	21.2	7.53
—	4.335	17.7	6.32
—	4.448	13.8	4.93
—	4.568	9.2	3.28
—	4.738	3.5	1.25
—	4.851	0.0	0.00
0.105	4.143	25.8	9.21
—	4.193	23.4	8.35
—	4.263	20.8	7.42
—	4.346	17.8	6.35
—	4.421	14.1	5.03
—	4.554	9.5	3.39
—	4.746	3.6	1.28
—	4.851	0.1	0.04

This definition is easily seen to coincide with that previously given by one of the present writers (K. Linderström-Lang l. c.):

$$\bar{r} = \frac{\sum c_r \cdot r}{\sum c_r} = \frac{\sum c_r \cdot r}{c_A} \quad (22)$$

where  $c_r$  denotes the concentration of an  $r$ -valent particle into which the ampholyte enters, (and which can contain both  $BH^+$ ,  $S^-$  and  $H^+$ ). The summation must extend over all particles into which the ampholyte enters.  $\sum c_r = c_A$ .

Similarly, we will now define the specific hydrogen ionisation of the ampholyte,  $\bar{h}$  by the equation

$$\bar{h} = \frac{\beta_H}{c_A} \quad (23)$$

We see from these equations that  $\bar{h}$  and  $\bar{r}$  need not be of equal magnitude. Only when the ampholyte is not capable of combining with other ions than the hydrogen ions is this the case.

#### b) Isoelectric and isoionic reaction.

The specific hydrogen ionisation of the ampholyte,  $\bar{h}$ , is found by determining the capacity to combine with acids and bases (vide infra) and the value  $pa_H$  at which  $\bar{h}$  is 0, generally identified with isoelectric reaction, need not therefore answer to an »isoelectric state« of the ampholyte. We will therefore define isoionic reaction as the value of  $pa_H$ ,  $pa_H^0$ , at which  $\bar{h}$  is 0, while isoelectric reaction is the hydrogen ion activity at which  $\bar{r}$  is 0.

It can now be shown, as we shall see more at length in a later publication, that if  $c_{BH^+}$  and  $c_{S^-}$  are great in comparison with  $c_A$  then  $\bar{h}$  will be 0 at the same hydrogen ion activity, independently of  $c_A$ , and all ionisation curves which at the same  $c_{BHS}$ , but with different  $c_A$ , express the relation between  $\beta_H$  and  $pa_H$  must therefore intersect at the same point, viz. where  $pa_H$  is equal to isoionic reaction, and  $\beta_H/c_A = \bar{h}$  is 0. This fact forms the basis for the experimental determination of isoionic reaction (cf. S. P. L. Sørensen »Studies on Proteins« l. c. p. 144). Fig. 2 shows graphically the results of a series of experiments from our material (Table 8).  $\beta_H$  is ordinate,  $pa_H$  abscissa. The three curves answering to the same ammonium chloride concentration (0.99 n) but different  $c_N$  (or  $c_A$ ) intersect at about the same point, at  $pa_H = 4.90$ , isoionic reaction. This can of course

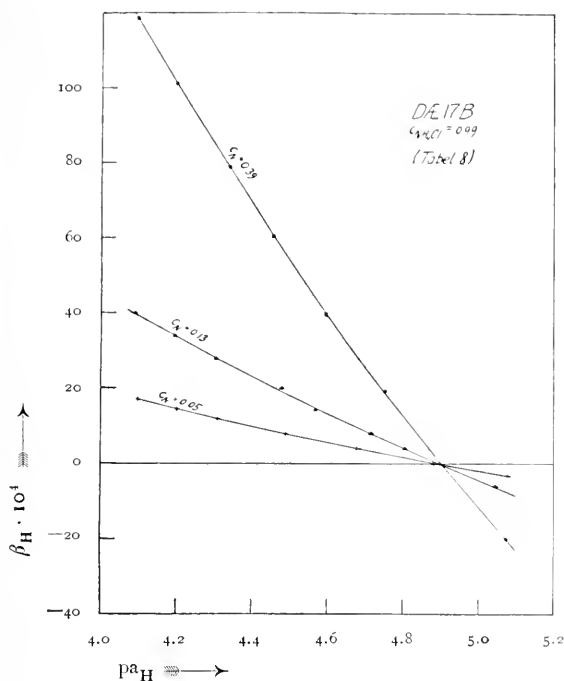


Fig. 2.

also be determined directly from each individual ionisation curve answering to the respective  $c_N$  (or  $c_A$ ) from the point of intersection with the  $p^a_H$  axis ( $\bar{h} = 0$ ), but, as mentioned on p. 11, it is possible that the salt BHS, if B be a weak base, may not contain absolutely equivalent quantities of B and HS. This will not affect the first method of determining isoionic reaction, but it will the last, as  $p^a_H^0$  will be found too great or too small, according as the salt contains a surplus of base or of acid not allowed for in the calculation of  $\beta_H$ . On the other hand, comparison of the two methods will afford information as to any surplus of acid or of base in the added salt. This comparison will be made later on, see p. 39—40.

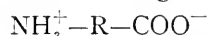
We shall in the following, in theory, be dealing for all essential purposes only with ampholytes, incapable of combining with other ions than the hydrogen ion, so that the isoionic and isoelectric reactions will coincide. We have nevertheless thought it only consistent to make the distinction. We shall later have occasion to deal with the problem on more general lines.

c) Qualities of ampholytes combining only with hydrogen ions.

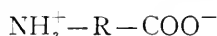
a. Alteration of isoelectric reaction with salt concentration.

As regards the alteration of isoelectric reaction with the concentration of salt, (see inter alia Michaelis<sup>1</sup>), Pauli<sup>2</sup>) the general treatment is rather complicated. We will here content ourselves with a simple instance.

A simple ampholyte with 1 amino group and 1 carboxyl group, which we will write according to Bjerrum<sup>3</sup>) as



is found in aqueous solution in the following forms:



If we now let the indices for activity  $a$ , activity coefficient  $f$  and concentration  $c$  denote the charge of the ion or molecule to which these quantities refer, we obtain:

$$\left. \begin{aligned} a_0; c_0 &= \frac{a_0}{f_0}; \text{ valency } 0; c_r \cdot r = c_0 \cdot 0 = 0 \\ a_1 &= k_1 \cdot a_0 \cdot a_H; c_1 = \frac{k_1}{f_1} \cdot a_0 \cdot a_H; \text{ valency } +1; \\ c_r \cdot r &= c_1 \cdot I = \frac{k_1}{f_1} a_0 \cdot a_H \\ a_{-1} &= k_{-1} \cdot a_0 \cdot a_H^{-1}; c_{-1} = \frac{k_{-1}}{f_{-1}} \cdot a_0 \cdot a_H^{-1}; \text{ valency } -1; \\ c_r \cdot r &= c_{-1} \cdot (-1) = -\frac{k_{-1}}{f_{-1}} \cdot a_0 \cdot a_H^{-1} \end{aligned} \right\} (24a)$$

$$\Sigma c_r \cdot r = a_0 \left( \frac{k_1}{f_1} \cdot a_H - \frac{k_{-1}}{f_{-1}} a_H^{-1} \right); \Sigma c_r = a_0 \left( \frac{1}{f_0} + \frac{k_1}{f_1} \cdot a_H + \frac{k_{-1}}{f_{-1}} \cdot a_H^{-1} \right)$$

whence:

$$\bar{r} = \frac{\frac{k_1}{f_1} \cdot a_H - \frac{k_{-1}}{f_{-1}} a_H^{-1}}{\frac{1}{f_0} + \frac{k_1}{f_1} a_H + \frac{k_{-1}}{f_{-1}} \cdot a_H^{-1}} \quad (24)^b$$

$\bar{h}$  is here equal to  $\bar{r}$ . (24) has been previously formulated on

<sup>1</sup> Die Wasserstoffionenkonzentrationen. Teil I. Julius Springer (1922).

<sup>2</sup> Kolloidchem. d. Eiweisskörper. Th. Steinkopff (1920).

<sup>3</sup> Zeitschrift f. physik. Chem. **104**, 147 (1923).

the basis of the classical dissociation theory by L. Michaelis<sup>1)</sup> who calls  $\bar{r}$  »die Ladungsgrad«. This term however, seems hardly suitable, as the ampholyte is charged even when  $\bar{r}$  is 0.

Isoelectric reaction is obtained by taking  $\bar{r}$  as = 0 and solving the equation thus produced for  $a_H$ . We find:

$$a_H^0 = \sqrt{\frac{k_{-1}}{k_1} \cdot \frac{f_1}{f_{-1}}} \quad (25)$$

Reckoning here according to the Debye-Hückel theory<sup>2)</sup> which, in the first approximation, only considers the interaction between the ionic charges, then  $a_H^0$  will not alter with the concentration of the ampholyte. If the ampholyte solution also contains salt, of the concentration  $c_E$ , then  $a_H^0$  will not alter with  $c_E$ .

For we can<sup>2)3)</sup> write in general:

$$\ln f_r = -1.18 r^2 \sqrt{\mu} \quad (18^\circ) \quad \log f_r = -0.512 r^2 \sqrt{\mu} \quad (26)$$

where  $f_r$  is the activity coefficient of any ion of the valency  $r$ , and  $\mu$  is determined by

$$\mu = \frac{1}{2} \sum c_i i^2 \quad (27)$$

in which expression  $c_i$  indicates the concentration of those ions in the solution of the valency  $i$ , and the summation extends over all positive and negative ions in the solution.

We therefore obtain for our ampholyte:

$$\begin{aligned} \log f_1 &= -0.512 (1)^2 \sqrt{\mu} = -0.512 \sqrt{\mu} \\ \log f_{-1} &= -0.512 (-1)^2 \sqrt{\mu} = -0.512 \sqrt{\mu} \end{aligned} \quad (28)$$

which shows that  $f_1 = f_{-1}$  at all ampholyte and salt concentrations. If on the other hand  $f_1$  and  $f_{-1}$  owing to specific interactions between the ions (cf. J. N. Brønsted)<sup>4)</sup> vary in different ways, then  $a_H^0$  will alter with the concentration.

$\beta$ . Influence of salt concentration on the shape of the ionisation curve.

Let us assume that the ampholyte is found in a solution with the concentration  $c_A$  (total concentr.) and that there is also salt in the solution, of the concentration  $c_E$ , which is large com-

<sup>1)</sup> Biochem. Zeitschr. **103**, 225 (1920).

<sup>2)</sup> Phys. Zeitschr. **24**, 185 (1923). **25** (1924).

<sup>3)</sup> N. J. Brønsted and K. La Mer: Journ. Amer. Chem. Soc. **46**, 555 (1924).

<sup>4)</sup> Journ. Amer. Chem. Soc. **44**, 877 (1922). **45**, 2898 (1923).

pared with  $c_A$ , so that the quantities of base or acid which must be added in order to vary  $\bar{r}$  are so small that  $f_1$  and  $f_{-1}$  will not depend on them.

If now we are on the acid side of isoelectric reaction, and if  $k_1$  and  $k_{-1}$  are of such a nature that we can disregard the term  $\frac{k_{-1}}{f_{-1}} \cdot a_H^{-1}$  in (24), then  $\bar{r}$  will be determined by:

$$\bar{r} = \frac{\frac{k_1}{f_1} \cdot a_H}{\frac{1}{f_0} + \frac{k_1}{f_1} \cdot a_H} \quad (29)$$

Solving this equation with regard to  $a_H$  we obtain:

$$a_H = \frac{f_1}{f_0 \cdot k_1} \left( \frac{\bar{r}}{1 - \bar{r}} \right) \quad (30)$$

If on the alkaline side of isoelectric reaction, and disregarding the term  $\frac{k_1}{f_1} \cdot a_H$  in (24) we obtain:

$$r = - \frac{\frac{k_{-1}}{f_{-1}} a_H^{-1}}{\frac{1}{f_0} + \frac{k_{-1}}{f_{-1}} a_H^{-1}} \quad (31)$$

which, when solved with regard to  $a_H$  appears as:

$$a_H = \frac{k_{-1} \cdot f_0}{f_{-1}} \left( \frac{\bar{r} + 1}{-\bar{r}} \right) \quad (32)$$

Assuming now that  $f_0$  is constant with varying salt concentration  $c_E$  because the sum of the charges in the amphoteric  $NH_3^+-R-COO^-$  is 0 (for the term amphoteric see H. N. K. Rørdam)<sup>1)</sup>, and taking the logarithm on either side of (30) and (32) and differentiating with respect to  $c_E$ ,  $\bar{r}$  being kept constant (which can, on alteration of  $c_E$ , be effected by adding small quantities of acid or base) we obtain:

Acid side of isoelectric reaction

$$\frac{\partial \log a_H}{\partial c_E} = \frac{\partial \log f_1}{\partial c_E} \quad \text{or} \quad \frac{\partial \text{pa}_H}{\partial c_E} = - \frac{\partial \log f_1}{\partial c_E} \quad (33)$$

Basic side

$$\frac{\partial \log a_H}{\partial c_E} = - \frac{\partial \log f_{-1}}{\partial c_E} \quad \text{or} \quad \frac{\partial \text{pa}_H}{\partial c_E} = + \frac{\partial \log f_{-1}}{\partial c_E} \quad (34)$$

<sup>1)</sup> »Studies on activity« Doctoral thesis, Copenhagen 1925.

which, inserted in (26), gives:

$$\frac{\partial \text{pa}_H}{\partial c_E} = 0.512 \frac{\partial \sqrt{\mu}}{\partial c_E} \quad \text{acid side} \quad \left( \frac{\partial \sqrt{\mu}}{\partial c_E} \text{ positive} \right) \quad (35)$$

$$\frac{\partial \text{pa}_H}{\partial c_E} = -0.512 \frac{\partial \sqrt{\mu}}{\partial c_E} \quad \text{basic side} \quad (36)$$

It is easy to see that the variation of  $\text{pa}_H$  with  $c_E$  as found is entirely independent of what value of  $\bar{r}$  we maintain constant, and that the variation must therefore be the same for all points of the ionisation curve. This again must consequently be displaced, to run parallel with itself, along the  $\text{pa}_H$  axis, when  $c_E$  is altered, but while the displacement on the acid side of isoelectric reaction ( $\bar{r}$  positive) will, on an increase of  $c_E$ , tend in the direction of increasing values of  $\text{pa}_H$   $\left( \frac{\partial \text{pa}_H}{\partial c_E} \text{ positive} \right)$  the displacement on the basic side will tend in the direction of decreasing  $\text{pa}_H$  values  $\left( \frac{\partial \text{pa}_H}{\partial c_E} \text{ negative} \right)$ .

Fig. 3 shows this graphically in the case of an ampholyte where  $k_1 = 10^4$  and  $k_{-1} = 10^{-9}$  (with the ordinary terms for the base and acid dissociation constants  $k_b$  and  $k_a$  this answers to  $k_b = 10^{-10}$  and  $k_a = 10^{-9}$ ).  $\text{pa}_H$  is abscissa,  $\bar{r}$  ordinate. O is isoelectric reaction, A and B are the points where  $\bar{r}$  is  $1/2$  or  $-1/2$  as the case may be. The fully drawn curve represents the relation between  $\bar{r}$  and  $\text{pa}_H$  at the salt concentration 0, the dotted curve that for salt concentration  $c_E$ .

In order to form some idea as to the order of magnitude of this dislocation, we can imagine for instance that  $c_E$  is 0.1 n and the salt KCl. It will then amount to  $\Delta \text{pa}_H = \pm 0.512 \Delta \sqrt{\mu} = \pm 0.512 \sqrt{0.1} = \pm 0.162$

The two parts of the  $\bar{r}$ - $\text{pa}_H$  curve thus undergo a parallel dislocation; we can however, in one sense, say that the curve as a whole is turned, since the axis X (see Fig. 3) round which the curve moves as a periodic function, will, on increasing the salt concentration, turn to the position  $X^1$ , the isoionic reaction remaining fixed (see (25) and (28)).

For further illustration of this, let us imagine the ampholyte capable of taking up  $n$  hydrogen ions per molecule, and suppose

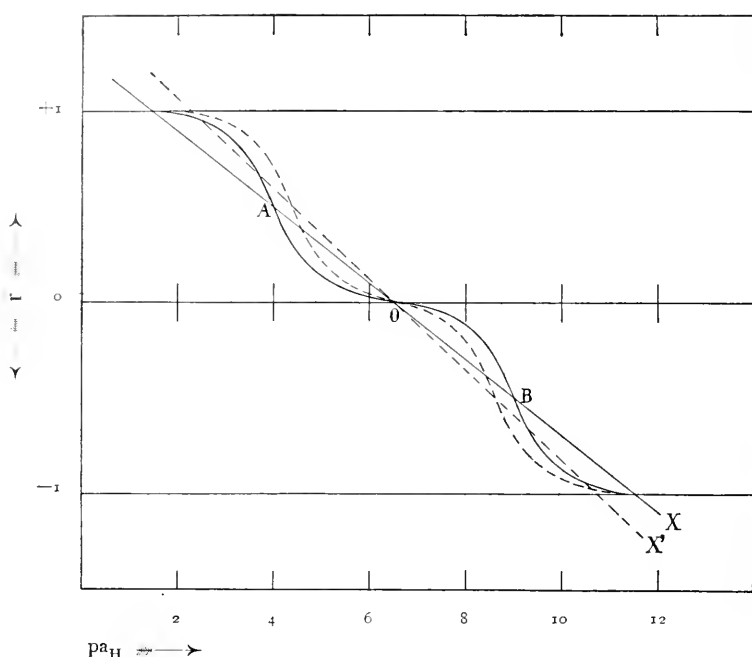


Fig. 3.

the equilibrium constants for the different dissociation stages to be so dissimilar that there are certain limited  $\text{pH}$  intervals where  $\bar{r}$  can with great approximation be determined by the equilibrium between two forms only (in the above example, for instance, between the uncharged and the singly charged positive form). We will here altogether disregard the fact that the true  $\text{pH}$  scale only suffices for the reproduction of two such typical dissociation intervals (Fig. 3).

For the equilibrium between an ampholyte ion of valency  $r$  (positive) and an ampholyte ion of the valency  $r+1$  the following mass action equation must hold good:

$$a_{r+1} = k_{r+1} \cdot a_r \cdot a_H \quad (37)$$

or, with  $a_{r+1} = c_{r+1} \cdot f_{r+1}$  etc.

$$c_r = \frac{a_r}{f_r} \quad (38)$$

$$c_{r+1} = a_r \cdot \frac{k_{r+1}}{f_{r+1}} \cdot a_H \quad (39)$$

whence, with  $\bar{r} = \frac{\sum c_r \cdot r}{\sum c_r}$  and  $c_r + c_{r+1} = c_A$  (as we have assumed the concentration of all other ions to be insignificant)



$$\bar{r} = \frac{r \cdot \frac{a_r}{f_r} + (r+1) \frac{a_r}{f_{r+1}} \cdot k_{r+1} \cdot a_H}{\frac{a_r}{f_r} + \frac{a_r}{f_{r+1}} \cdot k_{r+1} \cdot a_H} = \frac{r + (r+1) \frac{k_{r+1}}{f_{r+1}} \cdot a_H}{\frac{1}{f_r} + \frac{k_{r+1}}{f_{r+1}} \cdot a_H} \quad (40)$$

which, solved for  $a_H$ , gives

$$a_H = \frac{f_{r+1}}{f_r} \cdot \frac{1}{k_{r+1}} \left( \frac{\bar{r}-r}{(r+1)-r} \right)^1 \quad (41)$$

Taking as before the logarithm, differentiating with respect to  $c_E$  (under the same conditions as before) and keeping  $\bar{r}$  constant, and writing according to (26)

$$\begin{aligned} \log f_{r+1} &= -0.512 (r+1)^2 \sqrt{\mu} \\ \log f_r &= -0.512 (r)^2 \sqrt{\mu} \end{aligned} \quad (42)$$

we obtain

$$\frac{\partial \text{pa}_H}{\partial c_E} = 0.512 ((r+1)^2 - r^2) \frac{\partial \sqrt{\mu}}{\partial c_E} = 0.512 (2r+1) \frac{\partial \sqrt{\mu}}{\partial c_E} \quad (43)$$

where  $\frac{\partial \sqrt{\mu}}{\partial c_E}$  is positive.

The parallel dislocation of  $\bar{r}$ - $\text{pa}_H$  curve along the  $\text{pa}_H$  axis thus increases — with a definite increase of  $c_E$  — when  $\bar{r}$  is increased ( $\bar{r}$  being, in each  $\text{pa}_H$  interval between  $r$  and  $r+1$ ).

This is shown on Fig. 4 for an ampholyte where  $n=5$ . The dotted curves represent a higher salt concentration than the fully drawn lines. The relation between the dislocation of two successive dissociation curves (answering to equilibrium between  $r$ - and  $r+1$ -valent, or between  $r+1$  and  $r+2$  valent ions respectively) is determined by (43).

We see, as before, that the axis for the  $\text{pa}_H$ - $\bar{r}$  curve turns on the point of isoelectric reaction from position  $X$  to position  $X^1$  when  $c_E$  increases (Curve I).

The direction of the turn is such that  $\bar{r}$  at the same  $\text{pa}_H$  is greater the greater  $c_E$  is.

If we now take a quantity  $\varphi_{r+1}$  determined by

$$\varphi_{r+1} = \frac{f_{r+1}}{f_r} \cdot \frac{1}{k_{r+1}} \quad (44)$$

<sup>1)</sup> It should be pointed out that this equation (as well as (30) and (32) from (24a)) can be more simply derived directly from (37). The calculations have, however, been made in this manner in order to introduce the term mean valency, which is of importance in more complicated cases.

then it follows from (41) that when  $\bar{r} = r + \frac{1}{2}$

$$a_H = q_{r+1} \quad p_{aH} = -\log q_{r+1} \quad (45)$$

These values of  $p_{aH}$  are marked on the curve by the stippled ordinates and the letters A, B . . . F. If we now let the dissociation constants  $k_r$  for the different stages at constant salt concentration approach one another, then the various  $q_r$  will also approach one another, and the points A . . . F will close in to  $A^1, B^1 \dots$  (see Fig. 4, Curve II). The s-shaped equilibrium curves will overlap, and the periodic character of the  $\bar{r}$ - $p_{aH}$  curve will be effaced, giving place to a level appearance both in the non-varied and varied position, the latter being produced by a turn from the position Y to  $Y^1$  ( $c_E$  increases). At the same time we can, by suitable movement of A, B . . . F, bring  $A^1 B^1 \dots$  within the interval of the true  $p_{aH}$ -scale. This gives us then a typical ionisation curve for a polyvalent ampholyte.

The results of the foregoing may be summed up as follows: For a monovalent acid or base whose  $\bar{r}$ - $p_{aH}$ -curve has the shape indicated in the one half of Fig. 3, the salt effect gives rise to a parallel dislocation of the curve along the  $p_{aH}$  axis. In the case of a polyvalent ampholyte whose equilibrium constants for the different dissociation stages lie close together, the salt effect consists in a turning of the  $r$ - $p_{aH}$ -curve on the point of isoelectric reaction, and the direction of this turn is such that  $\bar{r}$  at the same  $p_{aH}$  increases with increasing  $c_E$  on the acid side of isoelectric reaction, but decreases with increasing  $c_E$  on the basic side of isoelectric reaction. If the axis of the  $\bar{r}$ - $p_{aH}$ -curve be specially a straight line, (answering to constant proportion between the  $q$ 's) then it will be straight at any concentration of salt.

Several of the points emphasised in the foregoing are well known (cf. Micaelis: Die Wasserstoffionenkonzentration, l. c. K. Kondo, l. c. Frisch, Pauli and Valko, l. c. E. J. Cohn l. c.). What we here wished to point out especially is the peculiar turning of the acid-base-combining curve (ionisation curve) with the salt concentration, a movement which is really apparent in the case of egg-albumin (see pp. 37 and 49—53) and can hardly be interpreted otherwise, or can at any rate be interpreted in this way.

On the basis of Debye-Hückel's theory (l. c.) and the

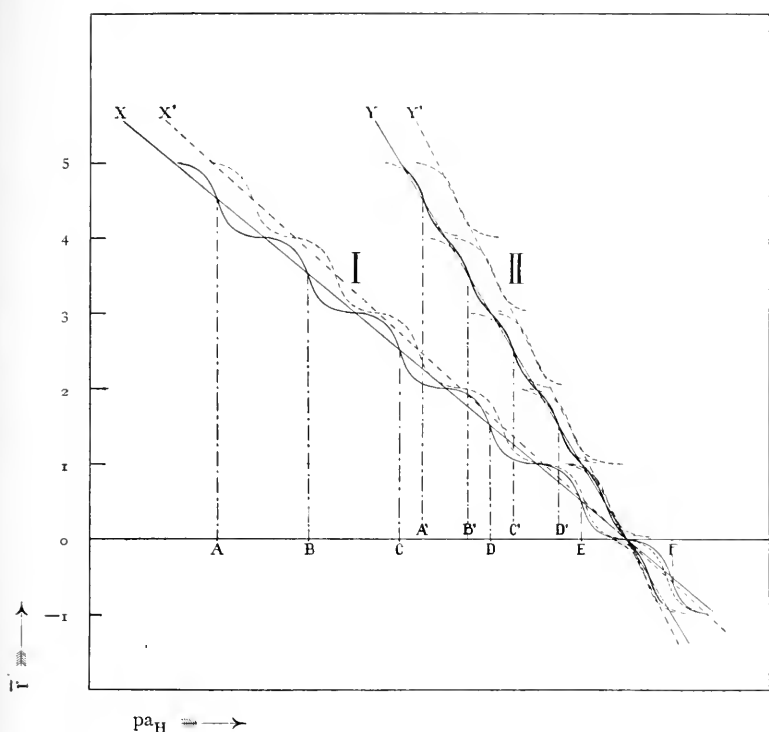


Fig. 4.

researches of Bjerrum<sup>1)</sup> as to the relation between the dissociation constants of dibasic acids, one of the present writers (l. c.) has, for a polyvalent ampholyte whose dissociation constants lie very close together, found by precisely similar arguments to those above applied, the following expression for  $\bar{r}$ :

$$\bar{r} = - \frac{1}{2(w + \frac{1}{q})} \ln \frac{a_H^0}{a_H} = \frac{p a_H^0 - p a_H}{0.8686 (w + \frac{1}{q})} \quad (46)$$

where  $a_H^0$  is isoelectric reaction,  $2q$  the number of acid and base groups in the ampholyte molecule, and  $w$  determined by

$$w = \frac{\epsilon^2}{2DakT(1 + \alpha a)} = \frac{0.355 \cdot 10^{-7}}{a(1 + \alpha a)} \quad (47)$$

in which last expression  $D$  is the dielectricity constant of the water,  $k$  the Boltzmann constant,  $T$  the temperature,  $\epsilon$  the elec-

<sup>1)</sup> Zeitschr. f. phys. Chem. **106**, 219 (1923).

tric elementary quantity,  $a$  the radius of the ampholyte molecule and  $z$  given by

$$z = \sqrt{\frac{4\pi\epsilon^2}{DkT} \sum i^2 n_i} = 3.297 \cdot 10^7 \sqrt{\mu} \quad (18^\circ) \quad (48)$$

$\mu$  as before being  $\frac{1}{2} \sum c_i i^2$  and  $n_i$  the no. of ions with valency  $i$  pr. cc.

For (46) to hold good, the following conditions must be approximately satisfied. The ampholyte ions must be spherical, their charge must be situated in the centre of the sphere, and the affinity of the hydrogen ion for the ampholyte molecule must be the same for all the  $2q$  »places« where it can be bound or given off.

Subject to these simplifying conditions, (46) becomes the equation of the axis in Fig. 4, and is therefore the limit equation to which the  $\bar{p}a_H$ - $\bar{r}$ -curve approaches when the dissociations constants move closer together. This will always happen when the radius of the ampholyte ions increases (see further in the work quoted) and an even  $\bar{r}$ - $\bar{p}a_H$ -curve following this equation must therefore be regarded as a simple result of the fact that the ampholyte ions are large (which, as far as we know, is the case with egg-albumin).

(46) is the equation of a straight line if we can, as above, disregard the concentration of the ampholyte ion in the expression for  $\mu$ , i. e. that  $c_E$  is large in proportion to  $c_A$ . For in such case  $\mu$  and thus also  $w$  will be independent of  $\bar{p}a_H$  and

$$\frac{1}{0.8686 \left( w + \frac{1}{q} \right)}$$

the constant direction tangent for the  $\bar{r}$ - $\bar{p}a_H$ -curve.

If on the other hand, in (48) (or in  $\mu$ ) we cannot disregard the term

$$\frac{1}{2} \sum c_r \cdot r^2$$

which is that part of  $\mu$  which arises from the ampholyte ions, the  $\mu$  will increase with decreasing  $\bar{p}a_H$ , because the mean valency of the ampholyte ions increases with increasing  $\bar{p}a_H$ .  $w$  will therefore (through  $r$ ) depend on  $\bar{p}a_H$ , and (46) will no longer be the equation of a straight line.

It can be shown, as we shall consider further in a later communication, that the equation:

$$\Sigma c_r \cdot r^2 = c_A \cdot r^2 \quad (49)$$

applies, with a fairly high degree of accuracy, save just in the vicinity of isoelectric reaction, where the contribution of the ampholyte to  $\mu$  in general is insignificant, when  $c_A$  has values of the order of magnitude here in question. When therefore we consider that  $c_A \cdot \bar{r}$  equivalents of an acid or base are required to give the ampholyte the mean valency  $r$ , it is easily understood that the part of  $\mu$  arising from the ampholyte salt must be

$$\mu_p = \frac{1}{2} (c_A \cdot \bar{r}^2 + c_A \cdot \bar{r} \cdot [e]) \quad (50)$$

where  $[e]$  is the numerical valency of the ion answering to the ampholyte in the ampholyte salt.

We thus obtain, for the total value of  $\mu$ ,  $\mu_T$

$$\mu_T = \frac{1}{2} \Sigma c_e \cdot e^2 + \frac{1}{2} c_A \bar{r} (\bar{r} + [e]) \quad (51)$$

where  $c_e$  or  $e$  as the case may be are the concentrations and valencies of other ions present ( $c_E$ ). When no other ions but the hydrogen ions are bound by the ampholyte, then  $\bar{r} = \bar{h} = \beta_H / c_A$  (se p. 11 and (23)) and therefore:

$$\mu_T = \frac{1}{2} \Sigma c_e e^2 + \frac{1}{2} \beta_H (\bar{h} + [e]) \quad (52)$$

whence, by means of (48), (47), and (46):

$$pa_H^0 - pa_H = \bar{h} \cdot 0.8686 \left( w + \frac{1}{q} \right)$$

$pa_H$  can be calculated without disregarding the contribution of the ampholyte to  $\mu$ , when  $pa_H^0$  and  $q$  are fixed. We shall have occasion to consider such calculations in the following.

It must be noted that we are here throughout assuming that  $a$ , the radius of the ampholyte ions, enters into  $w$  in the manner indicated in (47). This is also correct when we can disregard the radius of the ions surrounding any given ampholyte ion; in other words, when we can disregard  $\mu_p$  in the expression for  $\mu_T$  the ionic atmosphere in the solution being then formed solely of ions ( $c_E$ ) whose radii are small in comparison with  $a$ . This is taken for granted in formulating (46).  $a$  should however, properly denote the shortest distance to which the pair of ions can

approach one another. This, in the case of two ampholyte ions, is  $2a$ , and in cases where  $\mu_P$  is an essential part of  $\mu_T$ ,  $w$  will therefore be found too great.

As however, our application of the Debye formulæ through (46) and (47) is only intended to serve, and can only serve, as a numerical illustration of the views put forward generally in the foregoing, making no claim to any high degree of exactitude for the formulæ as here applied, we shall not go further into the details, which are at best very uncertain theoretically.

From (47) and (48) together it is immediately apparent that  $w$  decreases with increasing  $\kappa$  increasing  $c_E$ . (46) therefore shows that  $r$  at constant  $pa_H$  (and  $pa_H^0$ ) and constant  $q$  will be the greater as the concentration of salt increases. In this way, the turn, and true turning direction, of the limit curve will be represented by (46) and thus also those of the  $\bar{r}$ - $pa_H$ -curve.

d) Remarks on ampholytes capable of combining with other ions besides the hydrogen ions.

What is now the position if we assume the ampholyte to be capable of combining with other ions than the hydrogen ion? The assumption will in certain cases involve a complication of our formulæ, and does not appear to be strictly necessary, as the conditions in the ampholyte solutions we shall have to deal with can be illustrated, at any rate qualitatively, from the conception of interionic forces alone. It will therefore be only reasonable first of all to ascertain whether there is any experimental basis for the assumption in the cases we have to consider. The question is highly complex, and the experimental determination as to whether binding takes place or not is unfortunately very uncertain. If we know the activity coefficient of the ion  $I_1$  (or ions) which the ampholyte was capable of combining with, in the ampholyte solution, then a simple measurement of the activity of  $I_1$  would give some information as to the combined quantity. Unfortunately, we do not know this activity coefficient, and we have at the moment no means of ascertaining whether a reduced activity of  $I_1$  is due to interionic forces of some sort or to »chemical« combination<sup>1)</sup>. The question may be roughly formulated as follows:

<sup>1)</sup> See e. g. Northrop and Kunitz (l. c.).

The positive ampholyte ions, which we will for the moment consider as large, have an electric double layer arising from the fact that the ampholyte has taken up hydrogen ions. The inner side of this double layer is therefore composed of hydrogen ions, the outer of negative ions. Have then any of these negative ions passed through the double layer and penetrated into the ampholyte particle or not? In both cases, the activity of the negative ions will be less than their total concentration.

Since we have in the foregoing based our considerations on the Debye-Hückel theory, there might thus far be reason enough to calculate, on this basis, the mentioned activity coefficient of the ampholyte solution, and compare it with the experimental observations. It is very doubtful, however, whether the Debye formulæ will avail to give more than a picture of the conditions in solutions of polyvalent ions, even though their radius may be supposed to be sufficiently large. At any rate, a difference between the activity coefficient found and that calculated cannot with certainty be attributed to a combining of  $I_1$  and there is the further difficulty that the calculation and experimental control of the calculated  $f_1$  will naturally have to be made with solutions highly diluted in regard to  $I_1$  but concentrated with regard to the ampholyte, since practically the whole of  $\mu$  must arise from the ampholyte's contributions in order to show the desired effect as clearly as possible. In such solutions, it is doubtful whether the ampholytes we shall consider — egg-albumin — have the qualities (constant molecular weight for instance) which we have assumed for them in the foregoing.

We have nevertheless made a calculation from Frisch-Pauli-Valko's determination (l. c.) of  $f_{Cl}$  in egg-albumin-hydrochloric-acid solutions, taking as basis a mean ionic radius of  $10^{-7}$  and found that the calculated values of  $\log f_{Cl}$  are about half as great as those found, answering to a difference of 15 % in  $f_{Cl}$ . If we here insist on the applicability of the Debye formulæ — which we are forced to do for the time being — and assume that no other forces prevail between the chlorine ions and the protein ions than the forces from the ionic charges, then this difference must be interpreted as a combining of the chlorine ions with the egg-albumin ions. But if such other forces do exist — molecular cohesion forces — then the difference can be explained without assuming that any combination takes place.

There is much to suggest that such forces are present, and that they are independent of the valency of the protein ions. We shall not, however, go into this question here, though we wish to revert to it on a later occasion. For the time being, we shall consider the position in the light of the following facts:

The quantity of combined chlorine ions (calculated as the above mentioned difference multiplied by the concentration of chlorine ions) is, in all experiments where it has been measured with any degree of accuracy, insignificant in comparison with the quantity of combined hydrogen ions, save in the vicinity of isoionic reaction.

The experiments of Manabe and Matula<sup>1)</sup> have shown that the activity-reducing influence of egg-albumin on the chlorine ion at isoionic reaction is here practically the same as found by Frisch-Pauli-Valko at  $p_{aH}$  values between 3 and 4.

We think then, that there will be no great error involved by our assuming that:

1) The combining of chlorine ions — the purely chemical, if we wish to put it so — is, at all activities and all concentrations of chlorine ions, very slight in comparison with the combining of hydrogen ions. Even at low chloride concentrations, the ampholyte combines with its maximal quantity of chlorine ions, and this retains its constant value up to the highest chlorine ion concentrations (3 n).

2) The combining of chlorine ions is the same at all  $p_{aH}$  values between 4 and 5. (The same applies to all other ions present except the hydrogen ion, which, owing to its special qualities, forms an exception).

If these assumptions hold good, individually or together, then none of the formulæ previously given will prove invalid. ((46) and its resultant expressions). This is immediately apparent from the entire constancy of the quantity of ions bound. All that the chemical combining can do is to prevent the isoionic reaction from coinciding with the isoelectric, viz. in the case where, at isoionic reaction and every other  $p_{aH}$ , there are not equivalent quantities of combined positive and negative ions. Isoelectric, or more properly, isoionic reaction  $p_{aH}^0$  in (46) is however determined directly from the experiments, so that the position of the calculated curve (as well as its direction) is fixed.

<sup>1)</sup> Biochem. Zeitschr. **52**, 369 (1923).



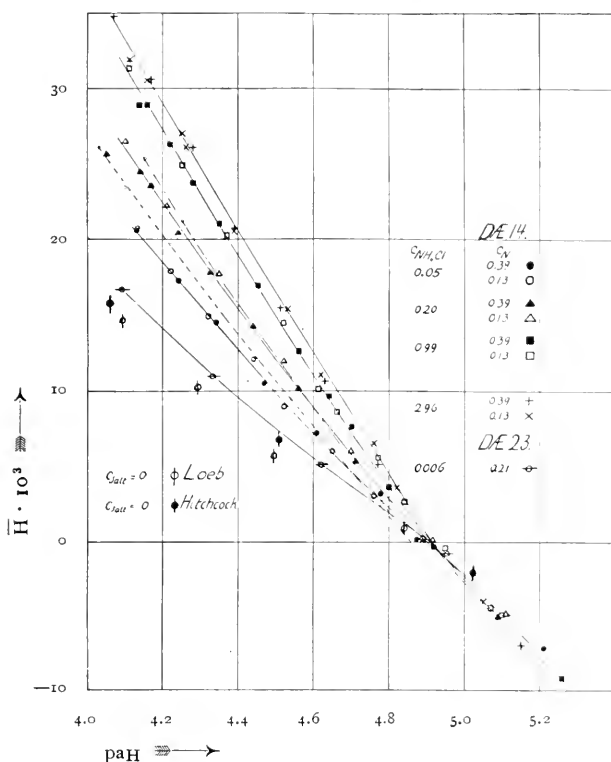


Fig. 5.

## 2) Survey of the Experimental Results.

Fig. 5 shows some of the experiments described on pp. 10 to 21 expressed in graphs,  $\bar{H} \cdot 10^3$  being the ordinate and  $p_{aH}$  the abscissa. The significance of the different curves and points will be seen from the figure. It is in the first place apparent that  $\bar{H} = \beta_H / c_N$ , within the limits of experimental error, is independent of  $c_N$ . Further, that the curves with increasing salt concentration become steeper, which, as mentioned, was to be expected; also, that they are very nearly straight lines, which there was also reason to expect would be the case. For purposes of comparison with the experiments previously made at this laboratory with ionisation of egg-albumin in ammonium sulphate solutions (Studies on Proteins) we have given 2 curves (Table II, p. 21) one dotted ( $c_{(NH_4)_2SO_4} = 0.06$ ) and one stippled ( $c = 0.354$ ) calculated from these earlier experiments,  $\bar{H} \cdot 10^3$  being equal to the

earlier  $(t-t^1)e$  where  $t$  and  $t^1$  are the number of cc n/1000 total or free sulphuric acid per 100 cc protein solution, while  $e$  is the number of mg-equivalents of protein nitrogen in the same volume (se Table 11 and reference there). As regards the direction of the ionisation curves, those of the ammonium chloride and ammonium sulphate experiments correspond, but isoionic reaction seems to lie rather more to the acid side for the last experiments, a point which we shall investigate in the following section.

Table 12 gives some measurements from publications by Loeb and Hitchcock (l. c.).

**Table 12.**

CN	$\text{CHCl} \cdot 10^3$	$Y \cdot 10^3$ corr.	pH	$\text{p}a_{\text{H}}$	$\bar{\text{H}} \cdot 10^3$	Observer
0.11	0	+0.10	4.80	4.84	+ 0.9	Loeb
0.11	0.5	0.60	4.45	4.49	5.4	
0.11	1.0	1.10	4.25	4.29	10.0	
0.11	1.5	1.60	4.05	4.09	14.4	
0.11	0	-1.27	5.63	5.67	-11.5	Hitchcock
0.11	1.0	-0.27	4.98	5.02	- 2.4	
0.11	2.0	+0.73	4.47	4.51	+ 6.6	
0.11	3.0	+1.73	4.02	4.06	+15.7	

The values directly found by Loeb and Hitchcock (those from Loeb's experiments read from the curve l. c. p. 93) are given in the first (1 % egg-albumin solution), second and fourth columns.  $\text{p}a_{\text{H}}$  was found from (20) and  $y$  corrected from  $\text{CHCl} \cdot \text{p}a_{\text{H}}$  curves by parallel dislocation along the  $\text{CHCl}$  axis, untill the point of intersection with the  $\text{p}a_{\text{H}}$  axis coincided with that found by us ( $\text{p}a_{\text{H}} = 4.90$ ). It will be seen that Hitchcock's egg-albumin contains surplus base. The values for  $\bar{\text{H}} \cdot 10^3$  thus found are marked off with the others in Fig. 5, and it will be seen that our experiments must be said to be in agreement with these when we consider that very small quantities of salt are present in Loeb's and Hitchcock's solutions. In those of the former none, in those of the other only that formed by neutralisation of the surplus base (abt. 0.001 n).

Frisch, Pauli and Valko's experiments were made at such low  $\text{p}a_{\text{H}}$  values that they lie outside the range we are con-

sidering,  $\text{p}a_{\text{H}} = 4-5$  (also, their egg-albumin was doubtless of a different composition from ours). It is very doubtful whether, in solutions of egg-albumin of more than  $\text{p}a_{\text{H}} = 4$  acidity, the egg-albumin can be trusted to retain its genuine qualities, and it is highly probable that the egg-albumin particles will, owing to the high valency, be decomposed into smaller particles. It is a well known fact that this decomposition, in the case of egg-albumin, is in all essentials irreversible, and accompanied by a denaturation. We shall therefore disregard all measurements with a more acid reaction than 4.

### 3) *Isoionic reaction.*

**Table 13.**

Isoionic reaction and ammonium chloride concentration.  $\text{p}a_{\text{H}}^0$ .

$\text{C}_{\text{NH}_4\text{Cl}}$	D. Æ. 14.		D. Æ. 17 B.	
	I	II	I	II
0.050	4.889	4.893	—	—
0.200	4.907	4.890	4.880	4.880
0.990	4.942	4.923	4.889	4.900
2.96	4.914	4.911	4.904	4.895

Table 13 gives the values for isoionic reaction: I, found as abscissa of the point of intersection of the  $\beta_{\text{H}}\text{-p}a_{\text{H}}$  curves with the  $\text{p}a_{\text{H}}$  axis, II, as abscissa of the point of intersection between the  $\beta_{\text{H}}\text{-p}a_{\text{H}}$  curves answering to protein concentrations  $\text{c}_{\text{N}} = 0.39$ , 0.13 and 0.05 (see p. 22 and Fig. 2). The agreement between the two modes of calculation must be said to be good. The greatest discrepancy is found in the experiment with D. Æ. 14,  $\text{c} = 0.99$ . As this experiment lies somewhat apart from the rest, and as the difference between the values (I) 4.942 and (II) 4.923 might tend to suggest that the ammonium chloride employed contained a small surplus of base, we have therefore recalculated the Y values in Table 5 by subtracting the constant quantity  $1.00 \cdot 10^{-4}$ , whereby the two methods are made to agree. These recalculated values are given in Table 5 a, and the values found from them for  $\beta_{\text{H}}$ ,  $\bar{\text{H}}$  and  $\bar{\text{h}}$  must thus be regarded as the correct ones.

As the mean values of the experiments with D. Æ. 14 and

D. Æ. 17 B we find the following values for  $\text{pa}_\text{H}^0$  and  $\text{a}_\text{H}^0$ , calculated according to method II (Columns 2 and 3, Table 14).

**Table 14.**

$\text{c}_{\text{NH}_4\text{Cl}}$	$\text{p}^0_{\text{H}}$	$\text{a}^0_{\text{H}} \cdot 10^6$	$\text{p}^0_{\text{H}}$ $\text{a}^0_{\text{H}} \cdot 10^6$ Mean		$\text{p}^0_{\text{H}}$ $\text{c}^0_{\text{H}} \cdot 10^6$ Mean	
	Method II					
0.05	4.893	12.79	4.898	12.65	4.857	13.90
0.20	4.885	13.03				
0.99	4.912	12.25				
2.96	4.904	12.47				

As these figures do not differ beyond the limits of error, we must for the present conclude that isoionic reaction may be regarded as very nearly independent of the ammonium chloride concentration, and we have therefore, in columns 4 and 5, noted the mean values for all salt concentration. For purposes of comparison,  $\text{p}_\text{H}^0$  and  $\text{c}_\text{H}^0$  are given in the last two columns, calculated according to the classical dissociation theory.

In previous experiments made at the Carlsberg Laboratory, the following value, independent of ammonium sulphate concentration, was found for the isoionic reaction of egg-albumin in ammonium sulphate solutions:

$$\text{pa}_\text{H}^0 = 4.844, \text{ corresponding to } \text{p}_\text{H}^0 = 4.803 \text{ and } \text{c}_\text{H}^0 = 15.74 \cdot 10^{-6}$$

As these figures do not agree very well with those now found, it may perhaps be of interest to endeavour to find the cause of this difference.

An investigation<sup>1)</sup> of the hydrogen ion activity measurement in strong ammonium sulphate solutions has shown that it is subject to a rather high degree of error, due to a liquid junction potential at the boundary surface between the ammonium sulphate solution and the 3.5 normal potassium chloride solution which forms the connecting liquid; a liquid junction potential the magnitude of which can only be estimated when measuring both with 1.75 and 3.5 n potassium chloride as connecting liquid.

In order to give an idea as to the importance of this source of error to the isoionic reaction (it has no effect on the form and

<sup>1)</sup> S. P. L. Sørensen and K. Linderstrøm-Lang: Comptes-rendus du Lab. Carlsberg **15**, No 6, (1924).

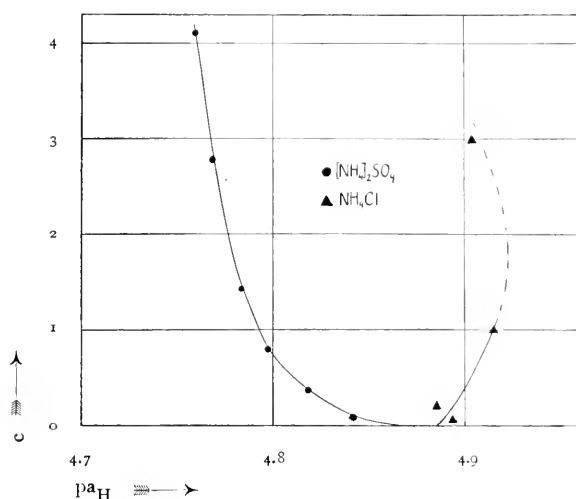


Fig. 6.

direction of the ionisation curve), we have calculated what  $p_{aH}^0$  ought to be if the liquid junction potential could be taken as equal to the difference between the liquid junction potentials with 1.75 n and with 3.5 n potassium chloride solution, a difference which is measurable in itself. We are however, as mentioned, well aware that the calculation cannot claim to be quantitatively exact, as has been shown by Bjerrum<sup>1)</sup> to be the case with dilute hydrogen ion solutions.

Table 15.

$C(NH_4)_2SO_4$	$p_{aH}$ uncorr.	$\Delta E$	$\Delta p_{aH}$	$p_{aH}$ corr.
0.06	4.844	0.0002	0.003	4.841
0.36	4.844	0.0015	0.026	4.818
0.80	4.844	0.0027	0.047	4.797
1.41	4.844	0.0035	0.061	4.783
2.79	4.844	0.0044	0.076	4.768
4.12	4.844	0.0049	0.085	4.759

In Table 15, the liquid junction potential  $\Delta E$ , the  $p_{aH}^0$  found, the correction for  $p_{aH}^0$ ,  $\Delta p_{aH}^0$ , and the corrected  $p_{aH}^0$  are indicated for different concentrations of ammonium sulphate. Fur-

<sup>1)</sup> Zeitschr. physik. Chem. **53**, 428 (1905).

ther, in Fig. 6, these corrected  $\text{pa}_\text{H}^0$  values are shown graphically together with those directly found in the ammonium chloride solutions. A small series of experiments has shown us that there is, as expected, no demonstrable  $\Delta E$  in ammonium chloride solutions up to 2 n.

It seems, from this figure, as if both curves would, at a sufficiently low concentration, approach very nearly to each other, and that in other words, isoionic reaction in salt-free solution lies between  $\text{pa}_\text{H} = 4.87$  and 4.89.

We shall not here go further into the cause of the variation in  $\text{pa}_\text{H}^0$  in ammonium sulphate solutions but refer to p. 25 and pp. 34—36. There is really nothing remarkable in the fact that ammonium chloride and ammonium sulphate should exhibit this difference, whether we assume that combining takes place, or that there are specific interactions, for the chlorine ion and the sulphate ion are both in regard to valency and other qualities extremely unlike, whereas the ammonium ion is very closely allied to the chlorine ion.

#### 4) *Calculation of the Ionisation Curves.*

Finally, in calculating the ionisation curves from the considerations given in the foregoing, we would once more point out the temporary character of all our ideas as to the constitution of protein solutions. Even though egg-albumin, in certain ammonium sulphate and ammonium chloride solutions about isoionic reaction, does behave as a high-molecular substance, with constant molecular weight and following the gas laws, there is yet much to suggest that this is not the case in salt-free solutions, or in solutions where the hydrogen ion concentration lies far from the point of isoionic reaction. How large this stable interval may be we can hardly say, and it is possible that we may already, in our mentioned experiments, have considerable divergence from these simple molecular conditions (cf. p. 39). Moreover, the applicability of formulæ such as (46) and others based on the Debye-Hückel theory will be to some extent limited, partly by the assumptions as to molecular structure which we are obliged to entertain, and partly from a fundamental uncertainty in the theory itself, which loses in value particularly when dealing with

ions of very high valency (cf. Bjerrum<sup>1)</sup>) and a higher salt concentration, even in the more general form in which it is here used, taking into consideration the radii of the ions (see e. g. Hückel<sup>2)</sup> and Bjerrum<sup>1)</sup>). Further, the possible presence of other forces between ions and molecules in solutions<sup>3)</sup> — not considered in the Debye theory — and the possibility of chemical combining of other ions than the hydrogen ion, which we have scarcely heeded here, oblige us, in conjunction with other features noted, to characterise the validity of our calculations as an ideal limit.

Subject to these reservations, however, we consider it justifiable to illustrate the position by means of formulæ such as (46) and those following. From the foregoing, it is evident that we thus regard the alteration in the direction of the ionisation curves as purely due to the action of inter-ionic forces.

On consideration of (46), (47) and (48) which we can, as  $\bar{r} = \bar{h}$  unite in the expression:

$$pa_H = pa_H^0 - h \left( \frac{0.308 \cdot 10^{-7}}{a(1 + a \cdot 3.297 \cdot 10^7 \sqrt{\mu})} + \frac{0.8686}{q} \right) \quad (53)$$

it will be seen that there are three quantities to be fixed,  $pa_H^0$ ,  $a$  and  $q$ . The molecular weight of egg-albumin, and the specific gravity of its particles, being known,  $\bar{h}$  can be found from the equation  $\bar{h} = \beta_H / c_A$  (see p. 11) and  $a$  can be determined (cf. K. Linderström-Lang<sup>4)</sup>).  $pa_H^0$  must be selected so as to coincide with that found by the experiments (cf. Fig. 5) while  $q$  must be determined from the direction of the ionisation curve at a single salt concentration. The formulæ thus found should then be correct for all the remaining salt concentrations.

We have chosen  $a = 2.21 \cdot 10^{-7}$  cm, answering to a molecular weight for egg-albumin of 35 000 in aqueous solution.  $c_A$  thus becomes  $= c_N / 357^5)$  and  $\bar{h}$  will be determined by:

<sup>1)</sup> Zeitschr. anorg. u. allg. Chem. **129**, 323 (1923).

<sup>2)</sup> Physik. Zeitschr. **26**, 93 (1925).

<sup>3)</sup> Comptes-rendus du Lab. Carlsberg **15**, No 4 (1923).

<sup>4)</sup> ibid. **15**, No 7 (1925).

<sup>5)</sup> These figures do not quite correspond to those given by Sørensen. As, however, they have been used before in the paper above quoted for similar calculations, and as there is no sure experimental basis for changing them, we will retain them here.

Table 16.

Calculated acid binding curves. Isoionic reaction  $4.898 \text{ (NH}_4\text{Cl)}$  or  $4.844 \text{ ((NH}_4\text{)}_2\text{SO}_4)$ .

$\beta_H \cdot 10^4$	$\bar{H} \cdot 10^3$	$h$	$\mu_P$	$\mu_T$	$\alpha \cdot 10^{-7}$	$q$	$q \cdot h$	$p_{aH}$
$\mu \text{ salt} = 0, c_N = 0.$								
0	9.52	3.40	0	0	0	0.1975	0.672	4.226
0	0	0	0	0	0	0.1975	0	4.898
$\mu \text{ salt} = 0, c_N = 0.210, \mu_P = \beta_H \cdot (h + 1) \cdot \frac{1}{2}.$								
50	23.81	8.50	0.024	0.024	0.511	0.1235	1.050	3.848
35	16.67	5.95	0.012	0.012	0.361	0.1355	0.806	4.092
20	9.52	3.40	0.005	0.005	0.221	0.1516	0.515	4.383
10	4.76	1.70	0.0015	0.0015	0.127	0.1670	0.284	4.614
0	0	0	0	0	0	0.1975	0	4.898
$\mu \text{ salt} = 0.006, c_N = 0.$								
0	9.52	3.40	0	0.006	0.248	0.1480	0.503	4.395
0	0	0	0	0.006	0.248	0.1480	0	4.898
$\mu \text{ salt} = 0.006, c_N = 0.210, \mu_P = \beta_H (h + 1) \cdot \frac{1}{2}.$								
50	23.81	8.50	0.024	0.030	0.571	0.1197	1.017	3.881
35	16.67	5.95	0.012	0.018	0.443	0.1285	0.765	4.133
20	9.52	3.40	0.0045	0.0105	0.338	0.1377	0.468	4.430
10	4.76	1.70	0.0015	0.0075	0.286	0.1436	0.244	4.654
0	0	0	0	0.006	0.248	0.1480	0	4.898



10	20.00	7.14	0.004	0.054	0.766	0.1099	0.785	4.113
8	16.00	5.71	0.0025	0.0525	0.756	0.1103	0.630	4.268
6	12.00	4.28	0.0015	0.0515	0.748	0.1105	0.473	4.425
4	8.00	2.86	0.001	0.051	0.744	0.1107	0.317	4.581
2	4.00	1.43	0.0005	0.0505	0.739	0.1109	0.159	4.739
0	0	0	0	0.050	0.737	0.1110	0	4.898

$\mu$  salt = 0.050.  $c_N = 0.130$ .

30	23.07	8.23	0.014	0.064	0.834	0.1071	0.881	4.017
25	19.23	6.86	0.010	0.060	0.807	0.1082	0.742	4.156
20	15.38	5.49	0.0065	0.0565	0.784	0.1091	0.599	4.299
15	11.54	4.12	0.004	0.054	0.766	0.1099	0.453	4.445
10	7.69	2.74	0.002	0.052	0.752	0.1104	0.302	4.596
0	0	0	0	0.050	0.737	0.1110	0	4.898

$\mu$  salt = 0.050.  $c_N = 0.390$ .

80	20.51	7.32	0.0335	0.0835	0.963	0.1026	0.751	4.147
60	15.38	5.49	0.0195	0.0695	0.869	0.1058	0.581	4.317
40	10.26	3.66	0.0095	0.0395	0.804	0.1082	0.396	4.502
20	5.13	1.83	0.003	0.053	0.759	0.1101	0.201	4.697
0	0	0	0	0.050	0.737	0.1110	0	4.898

Table 16 (cont.).

Calculated acid binding curves. Isoionic reaction  $4.898 \text{ (NH}_4\text{Cl)}$  or  $4.844 \text{ ((NH}_4\text{)}_2\text{SO}_4)$ .

$\beta_H \cdot 10^4$	$H \cdot 10^3$	$h$	$\mu_P$	$\mu_T$	$\alpha \cdot 10^{-7}$	$q$	$q \cdot h$	$p_{H_1}$
$\mu \text{ salt} = 0.056, c_N = 0.210, \mu_P = \beta_H (h + 1) \cdot \frac{1}{2}.$								
50	23.81	8.50	0.024	0.080	0.932	0.1036	0.881	4.017
35	16.67	5.95	0.012	0.068	0.860	0.1061	0.631	4.267
20	9.52	3.40	0.0045	0.0605	0.811	0.1080	0.367	4.531
10	4.76	1.70	0.0015	0.0575	0.791	0.1087	0.185	4.713
0	0	0	0	0.056	0.780	0.1093	0	4.898
$\mu \text{ salt} = 0.090, c_N = 0.210, \mu_P = \beta_H (h + 2) \cdot \frac{1}{2}, ((\text{NH}_4)_2\text{SO}_4).$								
50	23.81	8.50	0.0265	0.1165	1.126	0.0980	0.833	4.011
35	16.67	5.95	0.014	0.104	1.063	0.0996	0.593	4.251
20	9.52	3.40	0.0055	0.0955	1.019	0.1009	0.343	4.501
10	4.76	1.70	0.002	0.092	1.000	0.1014	0.172	4.672
0	0	0	0	0.090	0.989	0.1017	0	4.844
$\mu \text{ salt} = 0.106, c_N = 0.210, \mu_P = \beta_H (h + 1) \cdot \frac{1}{2}.$								
50	23.81	8.50	0.024	0.130	1.188	0.0964	0.819	4.079
35	16.67	5.95	0.012	0.118	1.132	0.0978	0.582	4.316
20	9.52	3.40	0.0045	0.1105	1.096	0.0988	0.336	4.562
10	4.76	1.70	0.0015	0.1075	1.081	0.0992	0.169	4.729
0	0	0	0	0.106	1.074	0.0994	0	4.808

50	23.81	8.50	0.024	0.230	1.581	0.0891	0.757	4.141
35	16.67	5.95	0.012	0.218	1.539	0.0897	0.534	4.364
20	9.52	3.40	0.0045	0.2105	1.512	0.0901	0.306	4.592
10	4.76	1.70	0.0015	0.2075	1.502	0.0903	0.154	4.744
0	0	0	0	0.206	1.494	0.0904	0	4.898
$\mu$ salt = 0.200. $c_N = 0.390$ . $\mu_p = \beta_H (h + 1) \cdot \frac{1}{2}$ .								
90	23.08	8.24	0.0415	0.2415	1.620	0.0885	0.729	4.169
80	20.51	7.32	0.0335	0.2335	1.593	0.0889	0.651	4.247
60	15.38	5.49	0.0195	0.2195	1.545	0.0896	0.492	4.406
40	10.26	3.66	0.0095	0.2095	1.509	0.0902	0.330	4.568
0	0	0	0	0.200	1.474	0.0907	0	4.898
$\mu$ salt = 0.531. $c_N = 0.21$ . $\mu_p = \beta_H (h + 2) \cdot \frac{1}{2}$ . $((NH_4)_2SO_4)$ .								
50	23.81	8.50	0.0265	0.5575	2.48	0.0796	0.677	4.167
20	9.52	3.40	0.0055	0.5365	2.43	0.0799	0.272	4.572
0	0	0	0	0.531	2.42	0.0800	0	4.844
$\mu$ salt = 0.989. $c_N = 0$ .								
—	23.81	8.50	—	0.989	3.28	0.0773	0.657	4.241
	0	0	—	0.989	3.28	0.0773	0	4.898
$\mu$ salt = 2.965. $c_N = 0$ .								
—	23.81	8.50	—	2.965	5.68	0.0683	0.581	4.315
	0	0		2.965	5.68	0.0683	0	4.898
$\mu$ salt = $\infty$ . $c_N = 0$ .								
—	16.67	5.95	—	$\infty$	$\infty$	0.0580	0.345	4.553
	0	0		$\infty$	$\infty$	0.0580	0	4.898

$$\bar{h} = \bar{H} \cdot 357$$

(see Tables 3 to 11).  $\text{pa}_H^0$  we have, for the ammonium chloride and potassium chloride experiments taken as equal to 4.898, for the ammonium sulphate experiments as 4.844.  $q$  is calculated from the experiment Table 3,  $c_{\text{NH}_4\text{Cl}} = 0.05$ , and it is found that  $q = 15$  will apply<sup>1</sup>). The number of acid and base groups in the egg-albumin molecule should therefore be 30. The uncertainty attaching to this figure is naturally very great (cf. Linderström-Lang l. c.) but when Loeb l. c., Hitchcock l. c., Cohn l. c., Frisch, Pauli and Valko l. c. find, at higher hydrogen ion activity, an ionisation far in excess of what should be the maximal from this figure, it may be taken as meaning that the egg-albumin molecule is decomposed, thus liberating more »base groups«.

We take, in other words:

$$a = 2.21 \cdot 10^{-7}$$

$$h = \bar{H} \cdot 357$$

$$\text{pa}_H^0 = 4.898 \text{ and } 4.844$$

$$q = 15$$

so that

$$\text{pa}_H = \text{pa}_H^0 - h \left( \frac{0.1395}{1 + 2.21 \cdot 3.297\sqrt{\mu}} + 0.0580 \right) \quad (54)$$

where  $\mu$  is determined by (52)

$$\mu = \mu_T = \frac{1}{2} \Sigma c_e \cdot e^2 + \frac{1}{2} \beta_H (\bar{h} + [e]) = c_{\text{NH}_4\text{Cl}} + \frac{1}{2} \beta_H (\bar{h} + 1) \quad (55)$$

for egg-albumin, ammonium chloride (or potassium chloride) and hydrochloric acid, and by

$$\mu = \mu_T = \frac{1}{2} \Sigma c_e \cdot e^2 + \frac{1}{2} \beta_H (h + [e]) = \frac{3}{2} c_{(\text{NH}_4)_2\text{SO}_4} + \frac{1}{2} \beta_H (\bar{h} + 2) \quad (56)$$

for egg-albumin, ammonium sulphate and sulphuric acid, where  $[e]$  is 2.

The calculated values are given in Table 16.  $\varphi$  is, after (54) given by

$$\varphi = \frac{0.1395}{1 + 2.21 \cdot 3.297\sqrt{\mu}} + 0.0580. \quad (57)$$

The last columns give the calculated  $\text{pa}_H$ .

<sup>1</sup> One of us has previously, from an ammonium sulphate experiment (Studies on Proteins l. c.) where  $c$  was 0.06 n, found  $q = 20$  by a calculation without regard to the protein ions' contribution to  $\mu$ . As we shall see, this particular experiment falls a little apart from the rest.

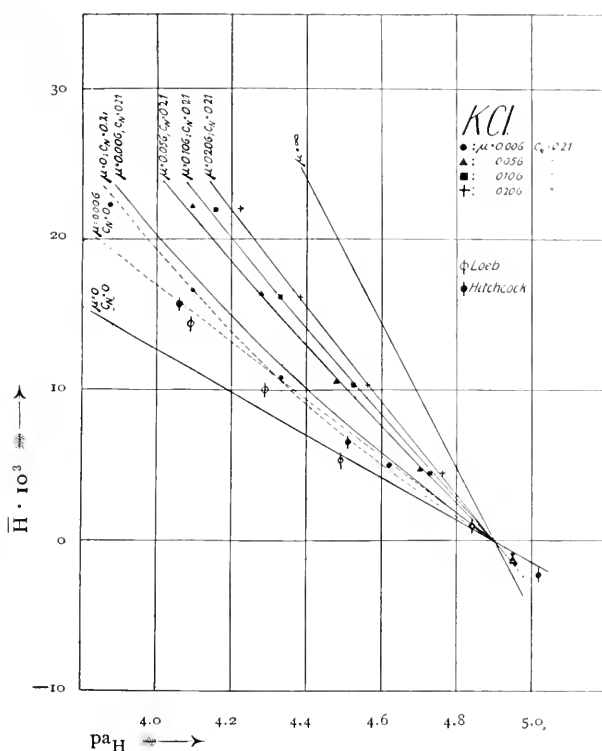


Fig. 7.

Figs. 7 to 12 show the results in graphical form.  $pa_H$  is the abscissa and  $\bar{H} \cdot 10^3$  ordinate (second column Table 16). The points marked all answer to values experimentally found (Tables 3—11) whereas all the curves are calculated. If therefore we wish to ascertain the agreement between theory and experiment, we must compare, in the figures, the position of the points and curves answering to the same salt concentration and protein concentration. In each individual figure we have given, not only the particular calculated curve or curves for the salt concentration to which the experimental points refer, but also other calculated — dotted — curves answering to other salt concentrations, thus facilitating the general view of the curves and the way they turn.

In calculating the values given in Table 16, we have taken a series of suitable values for  $\beta_H$  and dividing by the desired  $c_N$  found  $\bar{H}$ , whence by multiplication by 357,  $h$ . When  $h$  is known, we can, at the desired salt concentration, calculate  $\mu_p$  and  $\mu_T$  from

the expressions given above, (52), (55) and (56), and thence again  $\alpha$  from (48) and  $\varphi$  from (57). Multiplying  $\varphi$  by  $\bar{h}$ , we then obtain the value which must be subtracted from  $pa_H^0$  to give the calculated value of  $pa_H$  answering to the assumed  $\bar{H}$  (or  $h$ ).

We have given some limit values and limit curves for  $c_N=0$ , namely, at the salt concentrations 0, 0.006, and  $\infty$ . At the higher salt concentrations, where  $\mu_T$  is great in comparison with  $\mu_P$  it is of no importance to the course of the curve what value we give  $c_N$ ; this is not the case however, at lower concentrations, and as the introduction of  $\mu_P$  in  $\mu_T$  must be regarded as a rough correction, limited in its accuracy partly by what has been said on p. 33 and partly by the manner in which  $\Sigma c_r r^2$  is calculated (see (49) p. 32) we have, at the lower salt concentrations, thought it well to show what would be direction of the curves if  $c_N$  (and therewith  $\beta_H$  but of course not  $\beta_H/c_N$ ) were insignificant. It is also of interest to determine these limit curves for  $c=0$  and  $c=\infty$  to see whether all the points experimentally found lie between them. In calculating these, we have selected a suitable  $\bar{H}$  value and then proceeded as before.

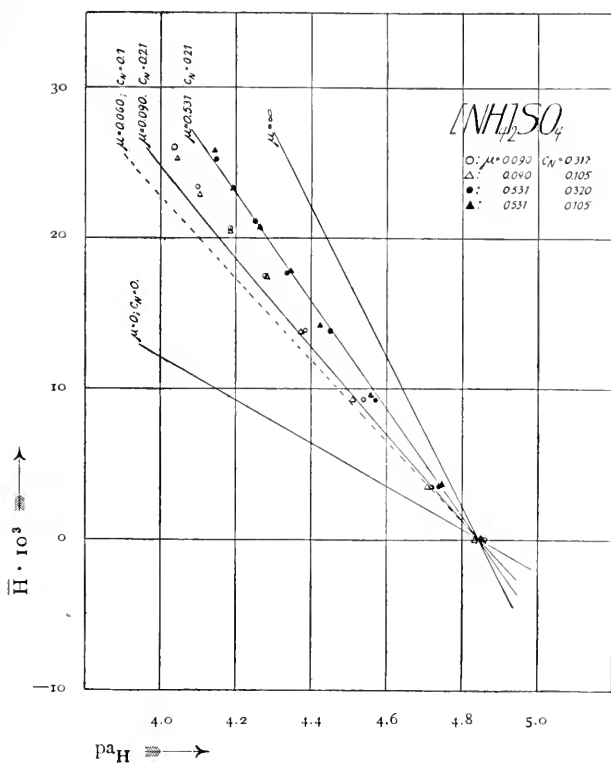
From this table, and these curves, it appears; that:

1) It is possible by means of a formula such as (46) or (54), determined solely by osmotic experiments and by the direction of a single ionisation curve (Fig. 8) to fix two limit curves for  $c=0$  and  $c=\infty$  between which all the points experimentally found will be situated.

2) It is possible with the aid of this formula to reproduce, in the main, both the shape of the ionisation curves and their turning, with varying salt concentration, and this in a manner rather satisfactory considering the premises.

3) The rectilinear character which the calculated curves exhibit when  $c_N$  is small compared with the concentration of salt, and which disappears when the reverse is the case, is, as already pointed out (l. c.) on the whole in agreement with the experiments, though the curves found appear to bend more at higher and less at lower salt concentrations than the calculated ones. The introduction of  $\mu_P$  in  $\mu_T$  seems, from the course of the curves at lower salt concentration, to be justifiable.

In this connection we would further observe: On the basic



side of isoionic reaction we find considerable divergence, which has not however been determined with certainty. We must here assume that the views as to the relation between the dissociation constants of egg-albumin on which (46) is based are not sound. As previously pointed out<sup>1)</sup> there is nothing surprising in this.

The accuracy of the expression for  $w$  (47) is doubtless greatly decreased at the high salt concentrations, but as  $w$  at the same time decreases with increasing  $\alpha$ , the error is thereby reduced.

As mentioned, also, the accuracy of (46) decreases with decreasing salt concentration, as the uncertainty of  $\mu_p$  here makes itself felt. It is therefore not surprising that the calculated curve for salt concentration 0.006 lies somewhat apart from that actually found, and that Loeb's and Hitchcock's points also lie somewhat outside.

At the ammonium chloride concentration 0.05 n ( $\mu = 0.05$ )

<sup>1)</sup> Comptes-rendus du Lab. Carlsberg **15**, No 7, p. 23 (1924).

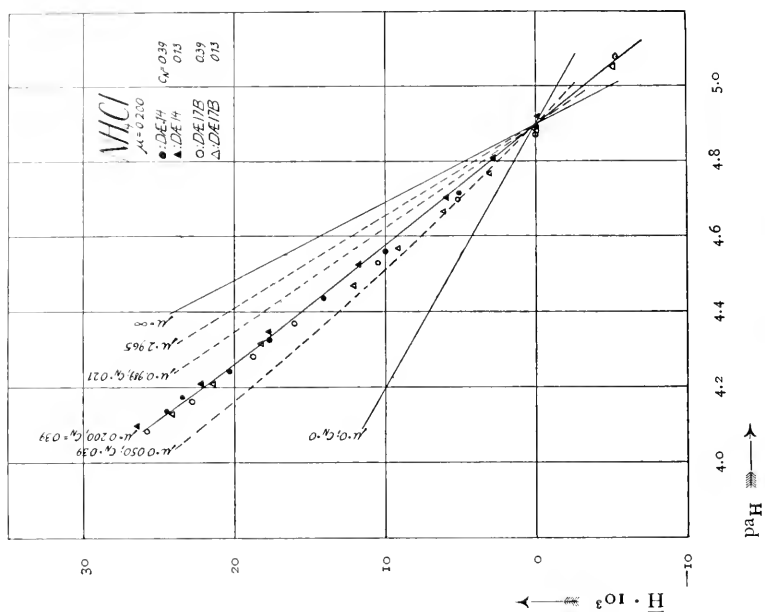


Fig. 9.

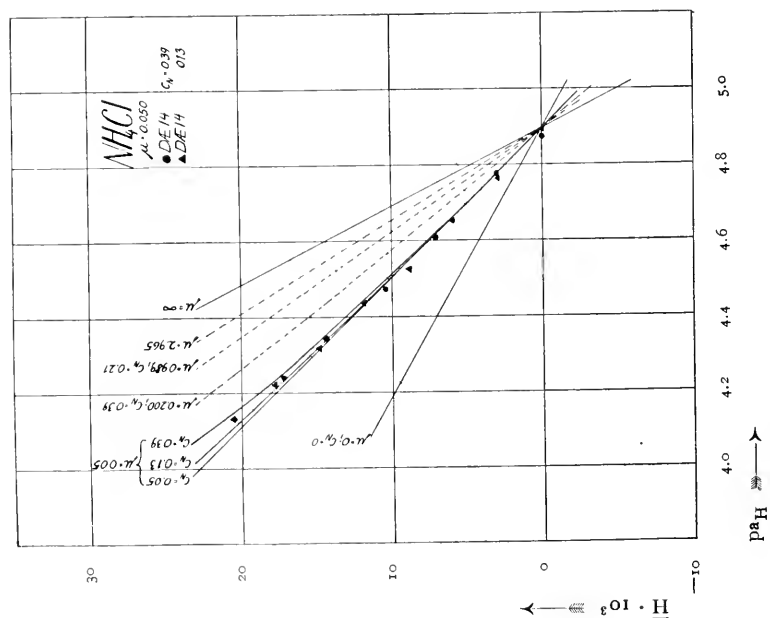


Fig. 10.



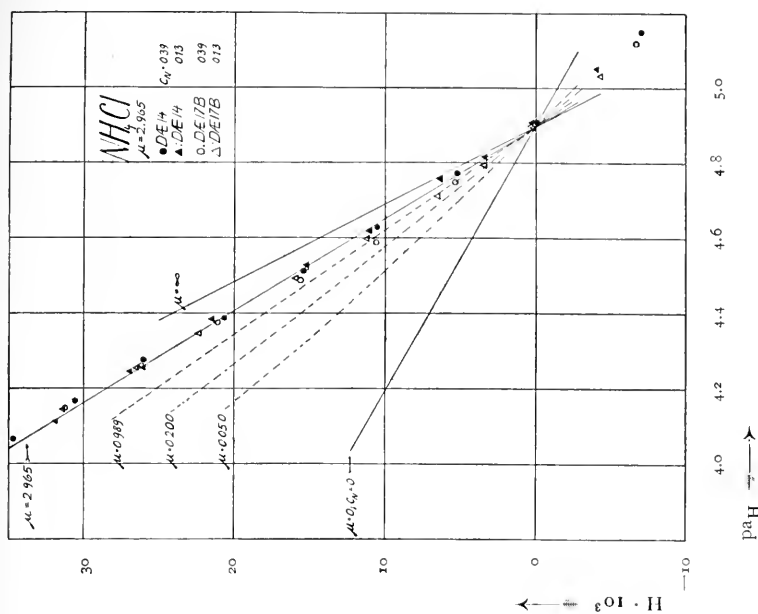


Fig. 12.

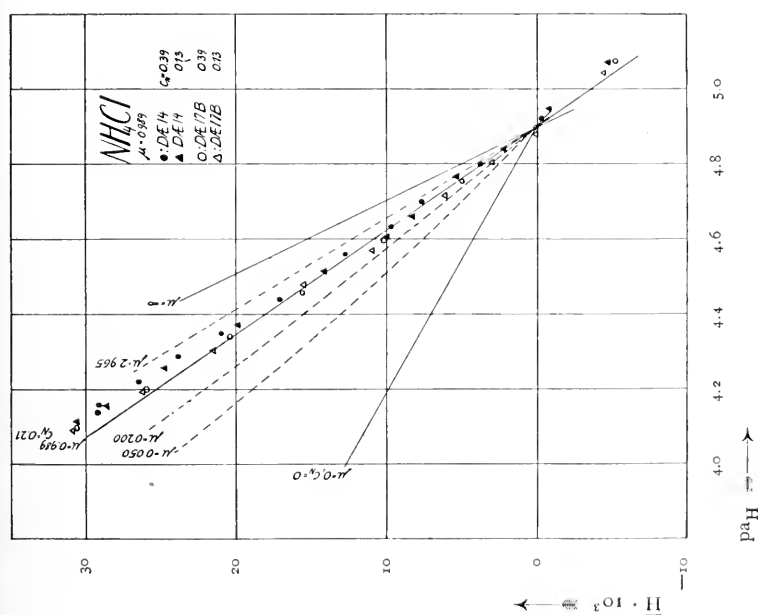


Fig. 11.

we find that  $\mu_P$  is already of subordinate importance, as the 3 curves answering to different  $c_N$  do not differ much one from another.

As regards the ammonium sulphate curves, the agreement at  $c_{(NH_4)_2SO_4} = 0.06$ ,  $\mu = 0.09$  is not so good; it is nevertheless evident that the effect of the double charge in the sulphate ion tends in the right direction, as the curve for  $\mu = 0.06$ , the same normality as the ammonium-sulphate solution, lies considerably farther from the points experimentally found.

## Summary.

### *Introduction.*

A description is given of the principle followed in the experimental determination of the ionisation of egg-albumin, its capacity to combine with acids and bases.

Egg-albumin is regarded as an ampholyte, and in accordance with J. N. Brønsted's definition of acids and bases, ampholytes are considered as substances capable of both taking up and giving off hydrogen ions. The theoretical treatment of the capacity of ampholytes to combine with acids (and bases) has been carried out on this basis.

### *Section A.*

Several experimental series are noted, comprising the determination of the activity coefficient of the hydrogen ion  $f_H$  in ammonium chloride solutions of different concentration.

### *Section B.*

The general method of experimental determination of the ionisation (capacity to combine with acid and bases) of egg-albumin in ammonium chloride and potassium chloride solutions is briefly described, and the results of the experiments are compared.

### *Section C.*

1) In a brief theoretical survey we have suggested that distinction should be made between isoelectric and isoionic reaction of an ampholyte, the former defined as the hydrogen ion activity (value of  $p_{aH}$ ) at which the mean valency of the ampholyte is 0, the latter as the hydrogen ion activity at which the quantity of acid or base combined with the ampholyte is 0;

or, as we prefer to express it, the hydrogen ion activity at which the specific hydrogen ionisation of the ampholyte is 0. If the ampholyte does not combine with other ions than the hydrogen ion, then isoelectric and isoionic reaction coincide. Isoionic reaction is determined by acid-combining experiments. The principle of this determination is briefly described.

A theoretical investigation of the alteration with salt concentration of both isoelectric (isoionic) reaction and the shape and direction of the ionisation curves is made, with regard to ampholytes capable only of combining with hydrogen ions, on the basis of the Debye-Hückel formulæ and Linderström-Lang's theory for the ionisation of polyvalent ampholytes of simple type. It is shown that the salt effect, in accordance with the theory, and in qualitative agreement with the experiments, consists in a turning of the ionisation curves, indicating the relation between the quantity of combined acid (specific hydrogen ionisation) and  $p_{aH}$ , and the turning of the curves, which leaves the isoelectric reaction unaltered, tends in such a direction that the quantity of combined acid at constant ampholyte concentration and constant  $p_{aH}$  increases with increasing salt concentration.

The possibility of chemical combining of other ions than the hydrogen ion is discussed.

2) Following on 1), a brief survey of the experimental results is given.

3) The isoionic reaction is found from the experimental material, and proved to be independent of the ammonium chloride concentration. As the mean of all determinations we have  $p_{aH}^0 = 4.898$  (isoionic reaction). The difference between this value and that formerly found for ammonium sulphate solutions (4.844) is discussed.

4) Finally, on the basis of the theory in Section 1), some simple calculations of the ionisation curves for egg-albumin are made, and it appears that the theory can reproduce the experimental results in a rough quantitative way when we assume that the egg-albumin molecule has a radius of  $2.21 \cdot 10^{-7}$  cm (answering to a molecular weight of 35000 in aqueous solution) and contains 30 acid and base groups.

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COMPTES-RENDUS

DES TRAVAUX

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16<sup>ME</sup> VOLUME.

No. 6

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COPENHAGUE

EN COMMISSION CHEZ H. HAGERUP

IMPRIMERIE DE THIELE

1926

Vol. 16, Nr. 5 est sous presse.

Prix: 3 Kr.



# ON THE STATE AND STABILITY OF COLLOID SOLUTIONS.

BY

K. LINDERSTROM LANG.

It is a well-known fact that certain high-molecular substances which, in conjunction with acids and bases, form salts soluble in water, behave abnormally in osmotic respects. The researches of Biltz and Vegesack<sup>1)</sup>, Biltz<sup>2)</sup>, Bayliss<sup>3)</sup>, Donnan and Harris<sup>4)</sup>, J. Loeb<sup>5)</sup>, N. Bjerrum<sup>6)</sup>, E Hammarsten<sup>7)</sup>, E. and H. Hammarsten<sup>8)</sup>, H. Hammarsten<sup>9)</sup> and Mc Bain and Salmon<sup>10)</sup>, Salmon<sup>11)</sup> have shown that the osmotic pressure, determined in various ways, of an electrolyte consisting of the high molecular ion  $I_n$  combined with any other, generally monovalent ion  $I_1$  (e. g. sodium ion or chlorine ion) often very nearly approaches the pressure which should be exerted by the number of "free" ions  $I_1$  determined by the electrometric method and calculated according to the classical dissociation theory. And this has been taken to mean that an extensive association, formation of micellæ, takes place. Attention has rightly been devoted more particularly to investigation of the substances for which the measured osmotic pressure appears to be less than would answer to the pressure of the free ions  $I_1$ . Substances having such qualities will in the following be said to be osmotically abnormal.

<sup>1)</sup> Zeitschr. f. physik. Chem. **68**, 357 (1909). **73**, 481 (1910).

<sup>2)</sup> Ibid. **77**, 91 (1911).

<sup>3)</sup> Proc. Roy. Soc. (B) **81**, 269 (1909).

<sup>4)</sup> Journ. Chem. Soc. **99**, 1554 (1911).

<sup>5)</sup> Proteins and the Theori of Colloidal Behavior (1922).

<sup>6)</sup> Zeitschr. physik. Chem. **110**, 656 (1924).

<sup>7)</sup> Biochem. Zeitschr. **144** (1924).

<sup>8)</sup> Arch. Kemi, Geol. og Mineralogi. Medd. Kngl. Vetensk. Akad. **8**, Nr. 27 (1923).

<sup>9)</sup> Biochem. Zeitschr. **147**, 481 (1924).

<sup>10)</sup> Journ. Amer. Chem. Soc. **42**, 426 (1920).

<sup>11)</sup> Journ. Chem. Soc. **117**, 530 (1920).

**Table 1.**

Pressure abnormality of thymonucleic acid salts. (After E. Hammarsten).

Anion	Kation	P (found)
		P (undiss.)
Thymonucleic ion . . . . .	H <sup>+</sup>	0.70
—	Na <sup>+</sup>	0.80
—	NH <sub>4</sub> <sup>+</sup>	1.20
—	(CH <sub>3</sub> ) <sub>3</sub> NH <sup>+</sup>	1.35
—	(CH <sub>3</sub> ) <sub>4</sub> N <sup>+</sup>	1.45
—	(C <sub>2</sub> H <sub>5</sub> ) <sub>3</sub> NH <sup>+</sup>	1.50
—	(C <sub>3</sub> H <sub>7</sub> ) <sub>3</sub> NH <sup>+</sup>	1.74

(P(undiss.) the pressure calculated for undissociated salt).

Another feature of the greatest interest is the effect discovered by E. and H. Hammarsten (l. c.) viz. that as shown in Table 1, the slight osmotic pressure only occurs when the ion answering to the high-molecular ion (but with opposite sign) ( $I_1$ ) is small. Hammarsten explains this effect, which we shall refer to in the following as the Hammarsten effect, by assuming that the small ions,  $I_1$ , are able to penetrate into the  $I_n$  ions, and thus become osmotically inactive, whereas large ions,  $I_1$ , are not capable of this. This explanation would be perfectly satisfactory if it were not that we have here to deal with substances which behave abnormally in osmotic respects; with regard to these however, it seems to me — as we shall see in the following pages — that there must be far different and more profound causes, and that Hammarsten's explanation, though perhaps necessary, is not sufficient.

If indeed we are to regard these solutions of colloid electrolytes as one-phase systems in internal equilibrium, — the point of view which will be maintained throughout the present paper — then in order to arrive at an understanding of the state of these osmotically abnormal electrolytes, we must altogether relinquish the classical dissociation theory and the thermodynamic methods associated therewith, endeavouring instead to make use of the general Gibbs equations<sup>1)</sup> and the

<sup>1)</sup> On the Equilibrium of Heterogeneous Substances. The Scientific Papers of J. W. Gibbs, Vol. I. Longmans Green & Co, London 1906.



thermodynamic principles developed in the papers of Lewis, Bjerrum and Brønsted.

By a thermodynamic method on these lines, we have, in the following, arrived at an explanation of the behaviour of abnormal electrolytes, which may be roughly expressed as follows:

Calling the activity of  $I_n$  and  $I_1$ ,  $a_n$  or  $a_1$  we obtain the following expression for the activity  $a_{n+1}$  of the salt consisting of  $I_n$  and  $I_1$ :

$$\left(\frac{1}{n} + 1\right) \ln a_{n+1} = \frac{1}{n} \ln a_n + \ln a_1, \quad (1)$$

the numerical valency of  $I_n$  and  $I_1$  being assumed to be  $n$  or  $1$  respectively.

The condition which must be satisfied if our electrolyte solution is to be stable as regards formation of new phases differing only infinitesimally from the original will then be:

$$\left(\frac{\partial \ln a_{n+1}}{\partial c_{n+1}}\right)_{p,T} \geq 0 \quad (2)$$

If this condition be not satisfied, then there is no possibility of the phase existing for any length of time. (2) is one of a series of partial stability conditions valid for each of the independent components which go to make up the phase, and each one of them must be satisfied if the phase is to be stable in the mentioned respect. As (2) then only holds good with regard to the independent components in the electrolyte solution, and as the following condition must always be satisfied:

$$c_n \cdot n = c_1 \quad (\text{condition of electro-neutrality}) \quad (3)$$

where  $c_n$  and  $c_1$  are the concentration of  $I_n$  and  $I_1$  then neither

$$\left(\frac{\partial \ln a_n}{\partial c_{n+1}}\right)_{p,T} \geq 0 \quad (4)$$

nor

$$\left(\frac{\partial \ln a_1}{\partial c_{n+1}}\right)_{p,T} \geq 0 \quad (5)$$

will be a partial stability condition for the phase ( $c_{n+1} = c_n + c_1$ ). In other words, it is possible to imagine a state of things where (2) is satisfied, for instance, but not (4) since according to (1).

$$\left(\frac{1}{n} + 1\right) \frac{\partial \ln a_{n+1}}{\partial c_{n+1}} = \frac{1}{n} \frac{\partial \ln a_n}{\partial c_{n+1}} + \frac{\partial \ln a_1}{\partial c_{n+1}} \quad (6)$$

In this equation, the first term on the right side may be less than 0 as long as the second is so much greater than 0 that the left side of the equation becomes positive.

(We would here emphatically point out that the phase is not necessarily stable simply because (2) has been satisfied. It may be unstable in regard to the formation of phases whose composition ultimately differs from the original, but it may possibly be stable).

Such states as are represented by the possibility above noted and thus characterised by the expressions:

$$\left( \frac{\partial \ln a_{n+1}}{\partial c_{n+1}} \right)_{p,T} \geq 0 \quad (2)$$

and

$$\left( \frac{\partial \ln a_n}{\partial c_{n+1}} \right)_{p,T} < 0 \quad (7)$$

are, we consider, found in solutions of electrolytes which behave abnormally in osmotic respects; often, in solutions of colloid electrolytes generally. For (7) is capable of explaining, in conjunction with (2) and (5), the experimental results. The certainty of this result is connected with the justification of using the thermodynamic equations for the solution at all. We hope later to have an opportunity of discussing this question. In the present instance, we think it of interest to consider the subject from one point of view.

On further consideration of (3) it is evident that the fact which, in spite of (7), determines the stability of the phase, is the electric charges of the ions. We shall in the following have occasion to go further into this.

Valuable information in regard to the colloid solutions is afforded by the excellent work on soaps carried out by McBain and collaborators<sup>1)</sup>; these experiments will therefore be given special consideration here. And in this connection we will endeavour to formulate a theory for the state in certain colloid solutions explaining why an expression such as (7) can hold good — in other words, why colloid electrolytes behave abnormally

<sup>1)</sup> McBain and Taylor: Ber. d. deutsch. chem. Gesellsch., **43**, 321 (1910); McBain, Cornish and Bowden: Journ. Chem. Soc. **101**, 2042 (1912); Bunbury and Martin: ibid. **105**, 417 (1914); Laing ibid. **113**, 435 (1918); McBain and Taylor: ibid. **115**, 1300 (1919); See also Journ. Chem. Soc. **115**, 1300 (1919); **119**, 1369, 1669, 1374 (1921); **125**, 1971 (1924); **127**, 853 (1925).

in osmotic respects, and explaining also the Hammarsten effect; an explanation which in reality, as regards the latter point, approaches very nearly to that advanced by Hammarsten himself. In this theory, we shall be chiefly concerned with the enormous forces of cohesion which must be acting between the large ions  $I_n$  owing to their relatively slight volume and their great deformability, properties closely related to their chemical constitution, paying less attention to the possibility of formation of micellæ. We consider, for instance, that it is not unreasonable to assume that soap solutions, at  $90^\circ$  within an extensive range of concentration, (up to 0.8 n) may be regarded as molecularly disperse.

In Section A, the thermodynamic treatment will be carried out more precisely and in general. In Section B we shall give a brief calculation of the conditions, agreeing in all essentials with that of Hammarsten, and based on the classical dissociation theory. The difficulties here arising will be discussed. In Section C, the thermodynamic treatment of osmotically abnormal electrolytes will be dealt with, and Section D will contain a discussion of McBain's investigations and the theory associated therewith. Finally, in Section E, some few calculations based on Hammarsten's experiments will be given by way of illustration.

I take this opportunity of expressing my sincere thanks to Professor S. P. L. Sørensen for the encouraging interest he has evinced in my work. I have also to thank Professor N. Bjerrum very heartily for advice and valuable criticism.

## A. General Thermodynamic Theory.

### 1. *The relation between factors determining the state of a phase.*

Let us consider the following Gibbs' equation (l. c.) valid in general for a single phase with  $n$  components:

$$SdT - vdp + \sum_n m_i dm_i = 0 \quad (8)$$

where  $S$  is entropy,  $v$  the volume of the phase,  $p$  pressure,  $m_i$  the quantity by weight of each of the components of the phase and

$M_i$  the chemical potential of the same. At constant pressure and temperature it becomes

$$\sum_n m_i dM_i = 0 \quad (9)$$

in (8) or (9) we can now divide each of the weight quantities  $m_i$  by any number, e. g. the molecular weight of the component (with Index  $i$ ) as long as the corresponding  $M_i$  is at the same time multiplied by the same figure. Let us call the new chemical potential thus arising (per gram-molecule component),  $\mu_i$ .

We obtain then, for an aqueous electrolyte solution, the ions of which  $I_1, I_2, \dots, I_i, \dots, I_n$  are of the concentrations  $c_1, c_2, \dots, c_i, \dots, c_n$  expressed as no. of gram-ions per 1000 g water:

$$\sum_n c_i d\mu_i + 55.5 d\mu_{\text{water}} = 0 \quad (p, T, \text{constant}) \quad (10)$$

If none of the  $I_i$  ions can be formed from the others, then (10) will hold good irrespective of what chemical compounds, association products between the ions, may be in mutual equilibrium in the solution, as long as we understand by  $c_i$  (etc.) the total concentration of  $I_i$  (etc.) — determined by direct analysis — and by  $\mu_i$  the directly — e. g. electrometrically — measurable chemical potential of  $I_i$ .

For if there be any association product,  $I_x$ :

$$I_x = x_1 I_1 + x_2 I_2 + \dots + x_n I_n + x_v H_2O$$

with the true concentration  $c_x^1$  in the solution, and calling the true concentrations of  $I_1, \dots, I, c_1^1, \dots, c_i^1, \dots, c_n^1$ , (10) then appears as follows:

$$\sum c_i^1 d\mu_i + c_x^1 d\mu_x + (55.5 - c_x^1 x_v) d\mu_v = 0. \quad (11)$$

$\mu_x$  being the potential of the association product.

We have, however,

$$x_1 \mu_1 + x_2 \mu_2 + \dots + x_n \mu_n + x_v \mu_v = \mu_x \quad (12)$$

the general mass action equation, and also:

$$\begin{aligned} c_1 &= c_1^1 + x_1 c_x^1 \\ c_2 &= c_2^1 + x_2 c_x^1 \\ &\vdots \\ c_n &= c_n^1 + x_n c_x^1 \end{aligned} \quad (13)$$

so that by differentiation of (12)

$$x_1 d\mu_1 + x_2 d\mu_2 + \dots + x_v d\mu_v = d\mu_x \quad (14)$$

and inserting in (11) we obtain:

$$\sum (c_i^1 + x_i c_x^1) d\mu_i + 55.5 d\mu_v = 0 \quad (15)$$

which, together with (13) gives (10).

Following a well known method, we will define the osmotic coefficient  $\varphi$  and the activity coefficients  $f_i$  for the different ions  $I_i$  by the following equations

$$d\mu_v = - \frac{RT}{55.5} d\varphi \sum c_i \quad (p, T, \text{ constant}) \quad (16)$$

$$d\mu_i = RT d\ln a_i = RT d\ln c_i f_i \quad (17)$$

where  $a_i$  is the activity of  $I_i$  (etc.). These equations inserted in (10) give the three following expressions, which are identical throughout:

$$\sum_n c_i d\ln a_i = d\varphi \sum c_i \quad \text{or} \quad \sum_n \frac{da_i}{f_i} = d\varphi \sum c_i \quad (18)$$

$$\sum_n c_i d\ln f_i = d(\varphi - 1) \sum c_i \quad (19)$$

whence by the integration from  $\sum c_i = 0$  to  $\sum c_i = \sum c_i$

$$\sum_n \int_0^{c_i} c_i d\ln f_i = (\varphi - 1) \sum c_i \quad (20)$$

or

$$\sum_n \int_0^{c_i} \frac{da_i}{f_i} = \varphi \sum c_i \quad (21)$$

If  $t$  be the freezing point lowering of the solution,  $t^1$  the rise of boiling point and  $P$  the osmotic pressure measured in cm. water, then the following will be valid with a sufficient degree of accuracy in such cases as are here in question:

$$\varphi \sum c_i = 0.5380 \cdot t = 2.079 \cdot t^1 = 0.4038 \cdot 10^{-1} P \quad (22)$$

(18) to (22) will give the relation between the measurable quantities which for us characterise the state of the solution:  $c_i$ ,  $a_i$ ,  $t$ ,  $t^1$ ,  $P$ .

It is often advisable to use (10), letting  $c_i$  refer to the true concentrations  $c_i^1$  (see (11)) of the ions present. We then obtain, assuming that  $\sum c_i$  is small in comparison with 55.5

$$\sum c_i^1 d\mu_i + 55.5 d\mu_v = 0 \quad (23)$$

where  $\mu_1 \dots \mu_x \dots \mu_r \dots \mu_n$  are the potentials of all the independent components and their association products  $I_x$ ,  $I$ , etc.

<sup>1</sup>) Cf. N. Bjerrum: On the Activity Coefficient for Ions. Medd. K. Vetensk. Nobelinstit. 5, No. 16. (1919), and J. N. Brønsted: On the Solubility of Salts in Salt Solutions, Det kongl. Danske Vidensk. Selsk. mat. fysiske Medd. II, Nr. 10 (1919).

We then define another osmotic coefficient  $\varphi^1$  and other activity coefficients  $f_i^1$  by the equations

$$d\mu_v = - \frac{RT}{55.5} d\varphi^1 \Sigma c_i^1 \quad (24)$$

$$d\mu_i = RT d \ln a_i = RT d \ln c_i^1 f_i^1 \quad (25)$$

which, inserted in (23) give two expressions analogous with (20) and (21)

$$\Sigma \int_0^{c_i^1} c_i^1 d \ln f_i^1 = (\varphi^1 - 1) \Sigma c_i^1 \quad (26)$$

$$\Sigma \int_0^{c_i^1} \frac{da_i}{f_i^1} = \varphi^1 \Sigma c_i^1 \quad (27)$$

where, as before.  $\varphi^1 \Sigma c_i^1 = 0.5380t = 2.079t^1 = 0.4038 \cdot 10^{-4} P. (28).$

## 2. Conditions on which the stability of a phase depends.

We have in the foregoing considered the ions  $I_i$  as independent components of the electrolyte solution. There is however, one condition which they must fulfil, and which is of decisive importance to the problem here, namely:

$$\Sigma c_i \cdot i = 0 \quad (29)$$

where  $i$  is the valency of  $I_i$ . It will therefore be impossible to remove any finite quantity of an ion from the phase without at the same time removing an equivalent amount of an ion with the opposite sign. The electric forces would prevent this.

This condition (29) now affects the requirement as to stability in the following manner:

It can be shown (see J.W. Gibbs l. c. p. 100—115 cf. also e. g. J. N. Brønsted l. c. p. 34) that the condition for stability of a phase in relation to continuous variation of its qualities is that each of the following partial differential expressions satisfy the conditions noted:

$$\begin{aligned} \left( \frac{\partial M_1}{\partial m_1} \right)_{v, T, m_1 \dots m_n} &\geq 0 & \left( \frac{\partial M_1}{\partial m_1} \right)_{v, T, m_1 \dots m_{i-1}, m_{i+1} \dots m_n} &\geq 0 \\ \left( \frac{\partial M_n}{\partial m_n} \right)_{v, T, m_1 \dots m_{n-1}} &\geq 0 \end{aligned} \quad (30)$$

These conditions are always necessary, but are not always sufficient, to ensure the stability of the phase. In other words, the phase is always unstable where these conditions are not

satisfied, and may or may not be so when they are. For it may possibly be unstable in regard to the discontinuous formation of a new phase, differing finitely in its composition from the original phase, and it may, again, be stable. If the phase be unstable in this latter respect, then there need not be any formation of a new phase, as "passive resistance" may prevent it. If it be unstable in the first respect, (with regard to continuous alteration) then a very slight rate of reaction will no doubt be able to retard the formation of new phases for certain periods of time, but never prevent them altogether.

The difference between the two kinds of stability or instability may be illustrated most simply as follows:

We imagine a gas following van d. Waals' equation

$$\left(p + \frac{a}{v^2}\right)(v-b) = RT$$

As we know, this equation, below the critical temperature, is graphically represented by a curve of the appearance shown in the accompanying figure, where the pressure,  $p$ , is the ordinate, and  $m$ , the number of grammes of gas per unit of volume, is the abscissa. Temperature constant.

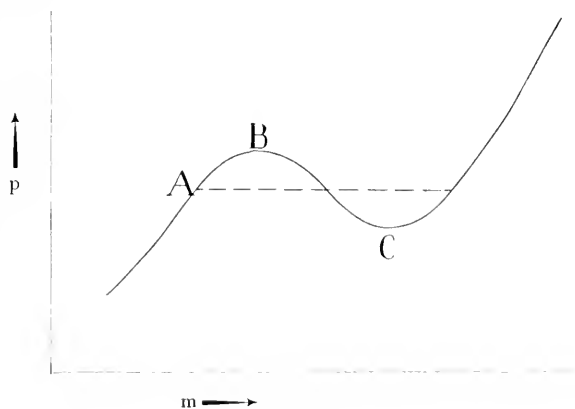


Fig. 1.

According to (8) we have for a phase containing but a single component:

$$-vdp + m dM = 0 \quad (T \text{ constant})$$

whence,  $v$  being constant,

$$v \left( \frac{\partial p}{\partial m} \right)_{v, T} = m \left( \frac{\partial M}{\partial m} \right)_{v, T}$$

Experience shows that the point A answers to a state of the phase in which it is unstable with regard to discontinuous formation of new phase, viz. condensed liquid. Owing to passive resistance, the phase can exist at a higher  $m$  than answers to this (undercooled vapour); in other words, there is the possibility of realising conditions answering to the line section AB. At point B, as we now see, we obtain

$$v \left( \frac{\partial p}{\partial m} \right)_{v, T} = m \left( \frac{\partial M}{\partial m} \right)_{v, T} = 0$$

and on the right side of B,

$$m \left( \frac{\partial M}{\partial m} \right)_{v, T} < 0$$

which is contrary to the condition for stability. States answering to the line section BC will not therefore be capable of realisation unless owing to a very slight rate of reaction, which is in accordance with experience.

Returning now to our electrolyte solution, we will restrict our observations to the simple case, where the solution contains only the ion  $I_n$  of valency  $n$  and the ion  $I_1$ , with valency 1, and for the rest, only such association products between these one with another as are in equilibrium reciprocally and also with  $I_n$  and  $I_1$ . We hope however, on a later occasion to return to a more general discussion of the problem.

According to (8) then, we have

$$m_1 dM_1 + m_n dM_n + m_v dM_v = 0 \quad (p, T \text{ constant}) \quad (31)$$

Comparing our phase now with another phase containing the same independent components, having the same pressure, and the same temperature, but whose values for  $m_1$ ,  $m_n$ ,  $m_v$ ,  $M_1$ ,  $M_n$  and  $M_v$  differ with the quantities  $\Delta m_1$ ,  $\Delta m_n$ ,  $\Delta m_v$ ,  $\Delta M_1$ ,  $\Delta M_n$  and  $\Delta M_v$ , which we assume to be very small, then the general stability condition, according to Gibbs, will be:

$$\Delta m_1 \Delta M_1 + \Delta m_n \Delta M_n + \Delta m_v \Delta M_v > 0 \quad (32)$$

If now  $m_1$  and  $m_n$  could be varied independently of each other, the partial stability conditions derived from this would then be

$$\left( \frac{\Delta M_1}{\Delta m_1} \right)_{p, T, m_n, m_v} > 0 \quad \text{or} \quad \left( \frac{\partial M_1}{\partial m_1} \right)_{p, T, m_n, m_v} \geq 0 \quad (33)$$

$$\left( \frac{\Delta M_n}{\Delta m_n} \right)_{p, T, m_1, m_v} > 0 \quad \text{or} \quad \left( \frac{\partial M_n}{\partial m_n} \right)_{p, T, m_1, m_v} \geq 0 \quad (34)$$

$$\left( \frac{\Delta M_v}{\Delta m_v} \right)_{p, T, m_1, m_n} > 0 \quad \text{or} \quad \left( \frac{\partial M_v}{\partial m_v} \right)_{p, T, m_1, m_n} \geq 0 \quad (35)$$



which may be directly seen for instance by setting in (32), in the case of (33),  $Im_n = 0$  and  $Im_v = 0$ . (Comparison of (33)–(35) with (30) shows that it is possible, among the values which must be kept constant in the partial differentiation, to set  $p$  in place of  $v$ . This however, only applies to condensed systems with more than one component).

If however, we call the molecular weight of  $I_1$  and  $I_n$ ,  $Q_1$  and  $Q_n$  and introduce the electro-neutrality condition (29) then we have

$$c_1 = c_n \cdot n \text{ or } \frac{m_1}{Q_1} = n \frac{m_n}{Q_n} \text{ or } Im_1 = n \frac{Q_1}{Q_n} Im_n \quad (36)$$

where  $n$  is taken as indicating the numerical valency of  $I_n$ .

(36) inserted in (32) gives us

$$\text{or } \left. \begin{aligned} & Im_n \left( n \cdot \frac{Q_1}{Q_n} IM_1 + IM_n \right) + Im_v IM_v = 0 \\ & Im_1 \left( IM_1 + \frac{1}{n} \cdot \frac{Q_n}{Q_1} IM_n \right) + Im_v IM_v = 0 \end{aligned} \right\} \quad (37)$$

The necessary and sufficient conditions for stability will therefore be:

$$\left( \frac{\partial \left( n \frac{Q_1}{Q_n} M_1 + M_n \right)}{\partial m_n} \right)_{p, T, m_v} \geq 0 \text{ or } \left( \frac{\partial \left( M_1 + \frac{Q_n}{Q_1} \frac{1}{n} M_n \right)}{\partial m_1} \right)_{p, T, m_v} \geq 0 \quad (38)$$

and, as before,

$$\left( \frac{\partial M_v}{\partial m_v} \right)_{p, T, m_1, m_n} \geq 0 \quad (35)$$

Taking here, as in the formulation of (10),  $m_v = 1000$  and dividing the  $m$ 's and multiplying the  $M$ 's by the molecular weights,  $M$  and  $m$  being thus replaced by  $\mu$  and  $c$ , we obtain, as the condition of stability,

$$\left( \frac{\partial (n\mu_1 + \mu_n)}{\partial c_n} \right)_{p, T} \geq 0 \text{ or } \left( \frac{\partial \left( \mu_1 + \frac{1}{n} \mu_n \right)}{\partial c_1} \right)_{p, T} \geq 0 \quad (39)$$

whereas neither

$$\left( \frac{\partial \mu_1}{\partial c_1} \right)_{p, T} \geq 0 \text{ nor } \left( \frac{\partial \mu_1}{\partial c_n} \right)_{p, T} \geq 0 \text{ nor } \left( \frac{\partial \mu_n}{\partial c_n} \right)_{p, T} \geq 0 \text{ etc.} \quad (40)$$

are necessary conditions of stability.

Introducing two terms  $\mu_{n+1}$  the potential of the salt, and  $c_{n+1}$  its concentration, defined by the equations

$$\left(1 + \frac{1}{n}\right) \mu_{n+1} = \mu_1 + \frac{1}{n} \mu_n \quad (41)$$

and

$$c_{n+1} = c_n + c_1 = c_1 \left(\frac{1}{n} + 1\right) = c_n(n+1) \quad (42)$$

(39) will then be altered to

$$\left(\frac{\partial \mu_{n+1}}{\partial c_{n+1}}\right)_{p,T} \geq 0 \quad \text{or} \quad \left(\frac{\partial \mu_{n+1}}{\partial c_n}\right)_{p,T} \geq 0 \quad \text{or} \quad \left(\frac{\partial \mu_{n+1}}{\partial c_1}\right)_{p,T} \geq 0 \quad (43)$$

and we have, as the condition of stability, (taking (17) into consideration, and with  $a_{n+1}$  as the activity of the salt)

$$\left(n \frac{\partial \ln a_1}{\partial c_1} + \frac{\partial \ln a_n}{\partial c_1}\right)_{p,T} \geq 0 \quad \text{or} \quad \left(\frac{\partial \ln a_1}{\partial c_n} + \frac{1}{n} \frac{\partial \ln a_n}{\partial c_n}\right)_{p,T} \geq 0 \quad (44a)$$

or

$$\left(\frac{\partial \ln a_{n+1}}{\partial c_{n+1}}\right)_{p,T} \geq 0 \quad \text{etc. etc.} \quad (44)$$

whereas neither

$$\left(\frac{\partial \ln a_n}{\partial c_{n+1}}\right)_{p,T} \geq 0 \quad \left(\frac{\partial \ln a_n}{\partial c_n}\right)_{p,T} \geq 0 \quad \text{nor} \quad \left(\frac{\partial \ln a_1}{\partial c_{n+1}}\right)_{p,T} \geq 0 \quad \text{etc.} \quad (45)$$

are necessary conditions for stability.

Having regard to (18) or (16) and (44a) we see that

$$\left(\frac{\partial q(c_1 + c_n)}{\partial c_n}\right)_{p,T} \geq 0 \quad \text{or} \quad \left(\frac{\partial q(c_1 + c_n)}{\partial c_1}\right)_{p,T} \geq 0 \quad (44b)$$

is also a necessary and sufficient condition of stability (cf. (10) and (35)).

## B. The Classical Dissociation Theory and Osmotically Abnormal Electrolytes.

### 1. Basis of the calculation.

We will take as the basis of our calculations the following equation:

$$\sum \int_0^{c_1} c_1^i d \ln f_1^i = (p^1 - 1) \Sigma c_1^i \quad (26)$$

expressing the main point of the classical dissociation theory as follows:

Assuming the existence of certain association products of the ions in an electrolyte solution in equilibrium with the ions themselves, it is possible to calculate the true concentrations of the ions and the association products in such a manner that they shall, for all dilutions, be proportional to, or more simply, equal to, the corresponding activities.

For instance, in a c n dilute acetic acid solution,  $f_H$  (without accent), the activity coefficient of the hydrogen ion, defined by (17) is determined by:

$$f_H = \frac{a_H}{c}$$

and is consequently very small. If on the other hand the true concentration of the hydrogen ions be  $c_H$ , and we assume an association of hydrogen ions and acetate ions to neutral acetic acid molecules, then  $f_H^1$  will, according to (25), be given by

$$f_H^1 = \frac{a_H}{c_H^1}$$

and is, as we know, very nearly constant ( $= 1$ ) in dilute solutions independent of  $c$ , as long as  $c_H^1$  is determined by the following equation:

$$c_H^1 = \frac{\sqrt{k^2 + 4kc} - k}{2} \quad (k \text{ being the diss. constant of the acetic acid})$$

So also with the acetate ion and the undissociated acetic acid, where we have

$$f_{Ac}^1 = \frac{a_{Ac}}{c_{Ac}^1} \text{ constant } (= 1) \text{ when } c_{Ac}^1 = \frac{1}{2} (\sqrt{k^2 + 4kc} - k)$$

and

$$f_{Edd}^1 = \frac{a_{Edd}}{c_{Edd}^1} \text{ constant } (= 1) \text{ when } c_{Edd}^1 = c - \frac{1}{2} (\sqrt{k^2 + 4kc} - k)$$

respectively.

Comparison with (26) shows that the constancy of  $f_i^1$  involves the disappearance of all integrals on the left side of the equation, which again makes  $q^1 = 1$ , or,

$$q^1 \sum c_i^1 = \sum c_i^1 \quad (46)$$

It is not essential to the validity of this equation that the integrals should severally disappear; but only that their sum should be 0; it is therefore important to point out that it is no criterion for the validity of the classical dissociation theory itself. Only when it is not satisfied, the classical theory no longer holds good.

Having regard to (28) the whole of this argument really amounts to the following:

Is it possible, by suitable selection of  $\Sigma c_i$  to make the terms

$$\text{or } \left. \begin{aligned} Q &= 0.5380 t - \Sigma c_i^1 \\ Q &= 2.079 t^1 - \Sigma c_i^1 \end{aligned} \right\} \quad (47)$$

etc., disappear? If not, then we cannot venture to employ the classical dissociation theory.

Assuming that it is possible electrometrically to determine the activity of one of the  $n$  ions, e. g.  $I_1$ , that, in accordance with the foregoing,  $a_1$  may be taken as equal to  $c_1^1$ , the true concentration of  $I_1$  (answering to  $f_1^1 = 1$ ), and forming the difference:

$$\text{or } \left. \begin{aligned} F &= 0.5380 t - a_1 = 0.5380 t - c_1^1 \\ F &= 2.079 t^1 - a_1 = 2.079 t^1 - c_1^1 \end{aligned} \right\} \quad (48)$$

etc., then a negative sign before  $F$  will mean that the classical dissociation theory does not hold good. For as  $\Sigma c_i^1 > c_1^1$ ,  $Q < F$ , and it is thus impossible to make  $Q$  disappear.  $\varphi^1 \Sigma c_i^1 = 0.5380 t$  (etc.) is often called the osmotically active concentration of the electrolyte, and  $F < 0$  therefore means that the osmotically active concentration is less than the concentration of the "free" ions  $I_1$ , or that for instance the osmotic pressure of the electrolyte is less than the pressure of the free ions  $I_1$ . After what has been said in the introduction, this characterises the osmotically abnormal electrolytes. In the case of these, the assumption of an association, however great, of the small and the large ions to micellæ will not suffice to explain the experimental results.

## 2. Results of the experiments.

On investigating the electrolytes which, according to E. and H. Hammarsten are osmotically abnormal, we encounter the following difficulty:

The determination of  $a_1$  the activity of one of the ions  $I_1$  in the electrolyte solution, is always a result of comparison with the activity of  $I_1$  in another electrolyte solution containing  $I_1$  (e. g. dilute hydrochloric acid in determination of hydrogen ions). The certainty of  $a_1$  therefore depends on the degree of accuracy with which the activity of  $I_1$  in the liquid used for comparison is

known. We will here disregard altogether the error arising from the liquid junction potential.

In most calculations of activities from electrometrical investigations, and also by Hammarsten, it has been presumed that — according to the classical dissociation theory — the concentration  $(I_1)_s$  of "free" ions  $I_1$  in the liquid for comparison can be expressed by

$$(I_1)_s = f_u (c_1)_s \quad (49)$$

where  $(c_1)_s$  is the concentration of  $I_1$  in the liquid for comparison and  $f_u$  the conductivity coefficient; also, that the concentration of the free ions  $I_1$  in the experimental liquid  $(I_1)$  can be calculated from the gas equation:

$$\pi = \frac{RT}{F} \ln \frac{(I_1)_s}{(I_1)} \quad (50)$$

where  $\pi$  is the potential difference between two  $I_1$ -electrodes in the comparison and experimental liquids respectively.

We know however, that (49) is not correct, and it is characteristic that even if the gas laws, and thus the classical dissociation theory also, hold good for the experimental liquid, the value of  $(I_1)$  according to (50) will be incorrect, as the same does not apply to the liquid for comparison.

We must therefore, consequently write, for the liquid for comparison:

$$(a_1)_s = (f_1)_s \cdot (c_1)_s \quad (51)$$

where  $(a_1)_s$  and  $(f_1)_s$  are the activity and activity coefficient of  $I_1$  in the liquid for comparison and determine  $a_1$  the activity of  $I_1$  in the experimental liquid, by the equation:

$$\pi = \frac{RT}{F} \ln \frac{(a_1)_s}{a_1} \quad (52)$$

which is exact. If now we identify the  $a_1$  thus found with  $(I_1)$  or, which amounts to the same thing, with  $c_1^1$  the true concentration of the free ions  $I_1$ , we are not thereby committing an error arising out of the conditions in the liquid for comparison, and thus immaterial to the experimental liquid, but only such error as it is precisely our business to investigate.

On this basis, then, all values arrived at by the classical dissociation theory will have to be re-calculated. The experiments we shall here consider are E. Hammarsten's investigations

with thymonucleinic acid and those of H. Hammarsten with protamin- and histon-chloride (l. c.). We shall thus have the hydrogen ion and the chlorine ion as  $I_1$  and our task will be to find  $a_{Cl}$  and  $a_H$  in the liquids for comparison used for these determinations.

$a_{Cl}$  in a 0.1 N potassium chloride solution, which is generally used as the liquid for comparison in measurement of chlorine ions, can be ascertained in various ways.

Bearing in mind that  $(a_{Cl})_s = 0.1 (f_{Cl})_s$  measurements of the freezing point lowering of potassium chloride solutions<sup>1)</sup> will, recalculating after (18) and setting the activity of the chlorine ion equal to that of the potassium ion, give:

$$\begin{aligned}\log (f_{Cl})_s &= -0.26 \sqrt[3]{0.1} = -0.1207 \\ (f_{Cl})_s &= 0.757 \\ (a_{Cl})_s &= 0.0757\end{aligned}$$

Measurements of the activity of potassium chloride solutions with calomel and potassium amalgam electrodes<sup>2)</sup> will, taking as before the activity of the chlorine ion as equal to that of the potassium ion, give:

$$\log (f_{Cl})_s = -0.265 \sqrt[3]{0.1} = -0.1230. \quad (f_{Cl})_s = 0.753; (a_{Cl})_s = 0.0753$$

Measurements of the chlorine ion activity, (Mac Innes and Parker<sup>3)</sup>, Lewis, Brighton and Sebastian<sup>4)</sup>) based on the same expression  $\log (f_{Cl})_s = -k \sqrt[3]{c_{KCl}}$  for  $(f_{Cl})_s$  give:

$$\log (f_{Cl})_s = -0.30 \sqrt[3]{0.1} = -0.1393; (f_{Cl})_s = 0.726; (a_{Cl})_s = 0.0726$$

Measurements of chlorine ion activity made by Manabe and Matula<sup>5)</sup> and Kondo<sup>6)</sup> are not sufficiently accurate for this purpose. Harned and Brumbaugh give<sup>7)</sup>

$$(f_{Cl})_s = 0.779 \text{ whence } (a_{Cl})_s = 0.0779$$

<sup>1)</sup> Noyes and Falk: Journ. Amer. Chem. Soc. **32**, 1011 (1910).

<sup>2)</sup> Noyes and McInnes: Journ. Amer. Chem. Soc. **42**, 239 (1920).

<sup>3)</sup> Journ. Amer. Chem. Soc. **37**, 1445 (1915).

<sup>4)</sup> Ibid. **39**, 2245 (1917).

<sup>5)</sup> Biochem. Zeitschr. **52**, 369 (1913).

<sup>6)</sup> Comptes Rendus du Lab. Carlsberg, **15**, 8 (1924).

<sup>7)</sup> Journ. Amer. Chem. Soc. **44**, 2729 (1922).

As it is difficult to give precedence to any single one of these measurements, I have taken the mean value:

$$(f_{\text{Cl}})_s = 0.754 \quad (a_{\text{Cl}})_s = 0.0754$$

For purposes of comparison it may be noted that measurements of conductivity give the following value for  $(\text{Cl})_s$  in the 0.1 n potassium chloride solution:

$$(\text{Cl})_s = 0.0860$$

so that the error involved by taking  $(a_{\text{Cl}})_s = (\text{Cl})_s$  is here rather considerable.

Consequently,  $a_{\text{Cl}}$  being the chlorine ion activity in any given electrolyte solution, and  $E$  the potential difference between a calomel electrode containing the same and a calomel electrode with 0.1 n potassium chloride, we have

$$\log a_{\text{Cl}} = E/0.0577 + \log 0.0754$$

while, with the measurements of conductivity as basis, we have

$$\log (\text{Cl}) = E/0.0577 + \log 0.0860$$

where  $(\text{Cl})$  answers to the concentration of free chlorine ions in the experimental liquid as calculated by Hammarsten. These two equations therefore give:

$$-\log a_{\text{Cl}} = -\log (\text{Cl}) + 0.057$$

$$\text{or} \quad p a_{\text{Cl}} = p_{\text{Cl}} + 0.057$$

$$a_{\text{Cl}} = (\text{Cl}) \cdot 0.877.$$

Similarly, we have for the hydrogen ion activity<sup>1)</sup>

$$-\log a_{\text{H}} = -\log (\text{H}) + 0.040$$

$$\text{or} \quad p a_{\text{H}} = p_{\text{H}} + 0.040$$

$$a_{\text{H}} = (\text{H}) \cdot 0.912$$

In this manner,  $a_{\text{Cl}}$  and  $a_{\text{H}}$  in Tables 2—5 have been calculated from the Hammarsten values for  $(\text{Cl})$  and  $(\text{H})$ , and in calculating  $F$ , inserted in (48).

<sup>1)</sup> S. P. L. Sørensen and K. Linderstrøm-Lang: Compt. rendus du Lab. Carlsberg 15, No 6 (1924).

**Table 2.**

Protamin chloride. Measurements of osmotic pressure.

g Prot · Cl pr. 1 Lit.	$a_{Cl} \cdot 10^3$ Inside	$a_{Cl} \cdot 10^3$ Outside	P found	P corr.	$\frac{P}{\cdot 0.4038 \cdot 1/10}$	$F \cdot 10^3$	$\Delta F \cdot 10^3$
3.96	8.77	0.09	218.2	222.7	8.99	+ 0.22	0.43
1.98	4.82	0.05	114.9	117.6	4.75	- 0.07	0.28
0.98	2.46	0.03	57.4	58.9	2.38	- 0.08	0.18
0.50	1.35	0.03	31.3	32.8	1.32	- 0.03	0.13

**Table 3.**

Protamin chloride. Freezing point measurements.

g Prot · Cl pr. 1 Lit.	$a_{Cl} \cdot 10^3$	t found	$t \cdot 0.538 \cdot 10^3$	$F \cdot 10^3$	$\Delta F \cdot 10^3$
11.47	24.2	0.048	25.8	+ 1.6	3.8
8.03	17.5	0.035	18.8	+ 1.3	3.5
5.74	12.8	0.024	12.9	+ 0.1	3.3

**Table 4.**

Histon chloride. Measurements of osmotic pressure.

g-Atom. N pr. 1 Lit. $10^3$	$a_{Cl} \cdot 10^3$ Inside	$a_{Cl} \cdot 10^3$ Outside	P found	P corr.	$\frac{P}{\cdot 0.4038 \cdot 1/10}$	$F \cdot 10^3$	$\Delta F \cdot 10^3$
98.0	4.39	0.12	88.0	93.9	3.79	- 0.60	0.24
48.6	2.28	0.04	44.5	46.3	1.87	- 0.51	0.17
19.3	0.97	0.04	17.2	19.1	0.77	- 0.20	0.12
9.5	0.53	0.05	7.2	9.7	0.39	- 0.14	0.10

**Table 5.**

Measurements of the osmotic pressure of thymonucleinic acid.

Molar conc.	$a_H \cdot 10^3$	P found	$\frac{P \cdot 0.4038}{\cdot 1/10}$	$F \cdot 10^3$	$\Delta F \cdot 10^3$
1.67	1.30	27.9	1.13	- 0.17	0.13
1.20	0.98	21.3	0.86	- 0.12	0.11
1.00	0.84	17.5	0.71	- 0.13	0.10



In determining the error upon  $F$ ,  $\Delta F$ , I have reckoned with an error of 1 millivolt in the potential measurement, 2 cm in that of the osmotic pressure and 0.005 degrees in the determination of freezing point. In the case of the last mentioned, H. Hammarsten does not give the method.

It will be seen from these tables how careful we must be in re-calculating electrometrical data to "true" concentrations. As for instance H. Hammarsten's chlorine ion concentrations lie some 10—15 % above the chlorine activities here given, the pressure anomaly calculated by him is considerably in excess of that here noted, which, in the case of protamin chloride, practically disappears.

In the case of histon chloride and thymonucleic acid on the other hand, it really seems as if the negative values found for  $F$  are not due to experimental error, and, as will be seen in the following, the agreement with freezing point measurements and the direct determinations of osmotic pressure suggest that the measurements are, thanks to exceptionally careful execution, really subject to far smaller errors than here calculated.

Substances for which  $F$  thus calculated is less than zero will in the following be called osmotically abnormal.

### C. Qualities of Osmotically Abnormal Electrolytes.

When, therefore, as pointed out by Hammarsten and referred to in the foregoing section, the classical dissociation theory fails us, we must look about for another mean of approach. E. and H. Hammarsten have, as noted in the introduction, regarded the problem as follows:

In an aqueous solution e. g. of thymonucleic acid, the thymonucleinate ions are of great volume. Owing to the smallness of the hydrogen ions, they are able completely or partially to penetrate — become enveloped in — the large negative ions, so that certain parts of their orbits will lie within the surface of the negative ions. Their osmotic activity is thus considerably reduced. There are two points which serve to support this view. In the first place, salts of meta-tungstic acid, which, though high-molecular is of small ionic volume, give, according to measurements by Sobolew, Rosenheim and Kohn, and Copaux normal

pressure; and further, the pressure of thymonucleinic acid compounds rises when the hydrogen ions are replaced by larger ions (the Hammarsten effect, see Table I, p. 2). It must be regarded as highly probable that such an enveloping effect is present, and plays an important part in the state of the electrolyte solution, but it is not sufficient to explain the behaviour of osmotically abnormal electrolytes, as it is, as far as I can see, very closely allied to the general ideas of association and complex formation in solutions. For if we imagine that at a given moment, the thermic movement in a solution ceases, then we must reckon all ions contained within the surface of the large ions at the moment as associated, complex-bound, and all the others as free (provided no forces are acting on them). Only these last will at the moment in question be osmotically active, but these are also the only ones active in an electrode equilibrium. These views, which must still be said to rest on the foundation of the classical dissociation theory, cannot therefore suffice to explain why the osmotic pressure should be smaller than the pressure of the free hydrogen ions.

The validity of the classical theory is closely connected with the validity of the gas laws for the solution, as the equation:

$$f_i^1 = \text{constant} = 1$$

precisely indicates that these apply to  $I_i$ ; when therefore, we wish to ascertain the cause of pressure anomalies, we must realise that it is to be looked for in some deviation from the gas laws. We might here first of all try the discrepancy caused by the electric forces between the charges of the ions; but, as we might expect, and as H. Hammarsten has endeavoured to show on the basis of the Debye-Hückel theory, these alone are not sufficient to explain the results obtained.

And in reality, the conditions are also far more pronounced, as will be seen from the following:

Taking (21) into consideration, and assuming that the components comprise only 3,  $I_1$ ,  $I_n$  and water, the equation appears as follows:

$$\int_0^{c_1} \frac{da_1}{f_1} + \int_0^{c_n} \frac{da_n}{f_n} = q' (c_n + c_1) = 0.5380 t = \text{etc.} \quad (53)$$

We form once more the difference,  $F$ , (cf. (48))

$$F = q \cdot (c_n + c_1) - a_1 = \int_0^{c_n} \frac{da_n}{f_n} + \int_0^{c_1} \frac{da_1}{f_1} - a_1 \quad (54)$$

whence, for osmotically abnormal electrolytes where  $F \leq 0$ ,

$$\int_0^{c_n} \frac{da_n}{f_n} + \int_0^{c_1} \frac{da_1}{f_1} - a_1 \leq 0 \quad (55)$$

$$c_n \cdot n = c_1$$

If now  $a_1$  and  $f_1$  be known as a function of  $c_1$  it is then possible to calculate the term

$$\int_0^{c_1} \frac{da_1}{f_1}$$

This is, in all Hammarsten's experiments, greater than  $a_1$ ; in other words:

$$\int_0^{c_1} \frac{da_1}{f_1} - a_1 > 0, \quad (56)$$

$f_1$  being, at all concentrations, less than 1, and the only possible solution of the expression (55) is therefore:

$$\int_0^{c_n} \frac{da_n}{f_n} < 0 \quad (57)$$

As  $f_n$  is always positive, this means that  $a_n$  in the concentration interval from 0 to  $c_n$  must, at any rate at a certain point of time, have decreased with increasing  $c_n$ ; in other words, at a certain concentration,

$$\left( \frac{\partial \ln a_n}{\partial c_n} \right)_{p,T} < 0 \quad (m_v = \text{constant} = 1000) \quad (58)$$

We have shown in a previous section that the existence of the phase need not therefore have been threatened, as long as throughout the entire concentration interval,

$$\left( \frac{1}{n} \frac{\partial \ln a_n}{\partial c_n} + \frac{\partial \ln a_1}{\partial c_n} \right)_{p,T} \geq 0 \quad (59)$$

Whatever function  $a_n$  may be, then, of the electrolyte concentration, there is always a possibility that a stable phase may exhibit abnormal osmotic con-

ditions. This possibility depends on what function of the electrolyte concentration  $a_1$  is. For the stability of the phase is determined by the relative magnitude of the two differential quotients

$$\frac{\partial \ln a_n}{\partial c_n} \quad \text{and} \quad \frac{\partial \ln a_1}{\partial c_n}$$

This possibility we now imagine to be realised in the osmotically abnormal electrolytes.

Less exactly, but more plainly, the contents of (57) may be expressed as follows. Defining by the equations

$$P_1 = RT \int_0^{c_1} \frac{da_1}{f_1} = \frac{10^4}{0.4038} \int_0^{c_1} \frac{da_1}{f_1} \text{ cm water}$$

$$P_n = RT \int_0^{c_n} \frac{da_n}{f_n} = \frac{10^4}{0.4038} \int_0^{c_n} \frac{da_n}{f_n} \text{ cm water}$$

the osmotic pressures  $P_1$  and  $P_n$  of the ions  $I_1$  and  $I_n$  respectively ( $P = P_1 + P_n$ , see (21) or (53) and (22)) we get from (57)

$$P_n < 0$$

i. e. the osmotic pressure of the ions  $I_n$  is negative. This fact explains more directly the behaviour of osmotically abnormal electrolytes (see pag. 14) but it must be pointed out that the quantities  $P_n$  and  $P_1$  thus defined are pure thermodynamic quantities and need not be identical with real pressures except in the case of dilute solutions of completely dissociated electrolytes.

Now it would be of the greatest interest to know the functions of the electrolyte solution ( $a$ ,  $\varphi$ ...) in the interval where  $\frac{\partial \ln a_n}{\partial c_n} < 0$ . This appears to be the case in the concentration interval investigated by Hammarsten. These experiments however are so difficult, and the substances employed are so hard to define, that the degree of accuracy obtainable at present can hardly be considered sufficient to make it worth while carrying out the calculation. We shall nevertheless, in Section E, consider a part of Hammarsten's numerical material.

Meantime, two things are evident from the foregoing:

Firstly, that the osmotic pressure of a solution at the concentration  $c$  depends in a peculiar fashion upon the state of the solution in the whole concentration interval  $0-c$ . This is a direct result of the fact that the gas laws do not apply to the substances dissolved.

And secondly, that an expression such as (58) can only be solved by assuming an extraordinary degree of interaction between the great ions  $I_n$ . For  $a_1$  does not exhibit similar dependence upon the concentration — though this must not be taken as implying that there is no interaction between  $I_1$  and  $I_n$  cf. p. 38.

Such interaction must be presumed to be due to powerful forces of attraction between the ions  $I_n$ ,  $f_n$ , which is a measure of the influence of these forces, is determined from (58), by

$$\frac{\partial \ln f_n}{\partial c_n} < - \frac{1}{c_n} \quad (60)$$

and  $f_n$  will therefore decrease to such a degree with increasing concentration, that only a very powerful mutual field of force between the ions  $I_n$  can explain this dependence.

We shall in the following section consider whether it is possible, from conceptions as to the structure of ions, to explain the presence of such forces. And this brings us to consideration of the theory of soap solutions.

## D. The Theory of Soap Solutions.

### 1) *The Experimental Results.*

In a system consisting of one salt with two monovalent ions  $I_s$  and  $I_1$  and water, the concentration of the salt per 1000 g of water being  $c$ , the following will, after (20) and (22), apply:

$$\int_0^c c d \ln f_s + \int_0^c c d \ln f_1 = (\varphi - 1) 2c = 2.079 t^1 - 2c \quad (61)$$

If now  $t^1$ , the rise of boiling point, be known, and thus also  $\varphi$  as a function of  $c$  for a sufficient concentration interval, then  $f_s \cdot f_1$ , the product of the activity coefficients of the two ions, can be calculated, since according to (19)

$$\begin{aligned} c d \ln f_s + c d \ln f_1 &= d (\varphi - 1) 2c \\ \ln f_s + \ln f_1 &= \int_0^c \frac{d (\varphi - 1) 2c}{c} \end{aligned} \quad (62)$$

we will now apply these equations to the salts of fatty acids after Mc Bain and Salmon (l. c.) disregarding the hydrolysis, which Mc Bain and his collaborators have shown to be of no importance. We shall in a later publication return to this question again.

Tables 6 and 7 give the results of their determinations of the rise of boiling point at  $90^\circ$  for potassium and sodium soaps of the fatty acid series.  $c$  is the no. of equivalents of soap per 1000 g water.

**Table 6.**

Rise of boiling point at  $90^\circ$  potassium soaps. (After Mc Bain og Salmon).

$c$	Stearate 18	Palmitate 16	Myristate 14	Laurate 12	Caprate 10	Caprylate 8	Caproate 6
0.2	0.10	0.12	0.13	0.15	0.16	0.17	0.17
0.5	0.17	0.19	0.23	0.26	0.31	0.35	0.38
0.75	0.19	0.23	0.27	0.30	0.42	0.48	0.53
1.0	0.20	0.24	0.28	0.32	0.52	0.60	0.66
1.5	0.16	0.21	0.25	0.31	(0.65)	—	—
2.0	0.18	0.27	0.32	0.48	(0.72)	—	—
3.0	0.23	—	—	1.02	—	—	—

**Table 7.**

Rise of boiling point of sodium behenate and potassium acetate. (Mc Bain og Salmon).

$c$	Potassium acetate 2	Sodium behenate 22
0.2	0.185	0.09
0.5	0.46	0.11
1.0	0.86	0.09
2.0	—	0.11

In Fig. 2, where the rise of boiling point is the ordinate and the reciprocal concentration the abscissa, the figures are shown as a graph, a number on a curve indicating how many atoms of carbon there are in the soap represented by the curve in question. With the exception of the curve for sodium behenate (No. 22) all the curves shown represent potassium soaps.

From the figures here given we have formulated the following approximate law for the relation between  $t^1$  or  $\varphi$  and  $c$ , valid for the concentration interval from 0.2 to 1 n:

$$2.079t^1 - 2c = (\varphi - 1) 2c = -Kc^{\frac{3}{2}} \quad \varphi - 1 = -\frac{K}{2}\sqrt{c} \quad (63)$$

from which, after (62)

$$\ln f_1 + \ln f_s = -3K\sqrt{c} = -K^1\sqrt{c} \quad (64)$$

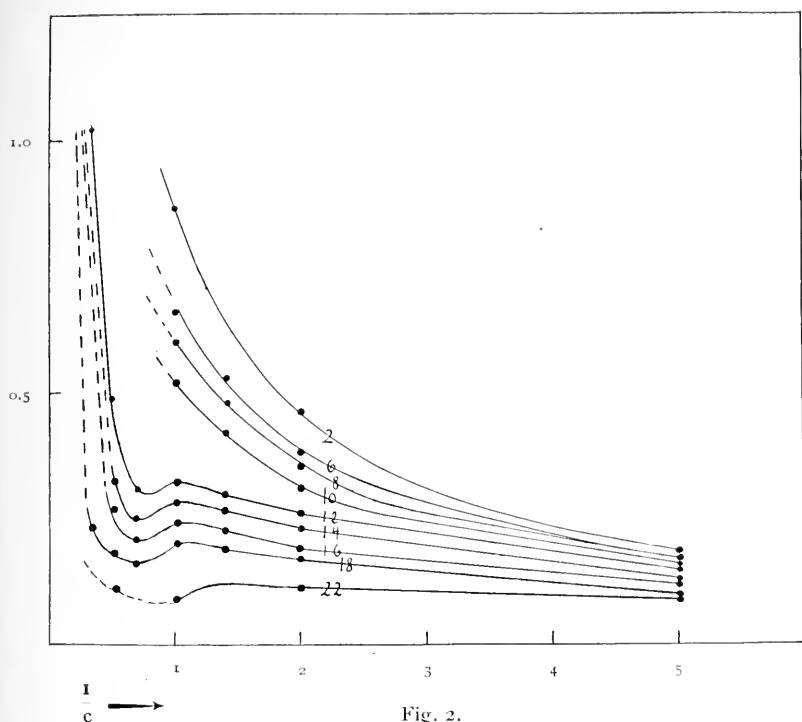


Fig. 2.

or, expressed in ordinary logarithms:

$$\log f_1 + \log f_s = -1.30 K/\bar{c} = -K^{11}/\bar{c}$$

The results arrived at by this method are given in Table 8, where  $K$ ,  $K^1$  and  $K^{11}$  have the same meaning as in (63) to (65).

The osmotic measurements are thus represented adequately, whereas there is only 1 measurement of the sodium ion activity, made by Salmon (l. c.) at  $90^\circ$  in a 1*n* sodium palmitate solution, and this measurement itself is very inaccurate, owing to the arrangement of the experiment, which permits a very high liquid junction potential — calculated after Henderson's formula to not less than 29 millivolts — which cannot be corrected with real certainty.

The measurements nevertheless give one the impression that the diffusion potential is the main source of error, and we calculate for 1*n* sodium palmitate solution,  $f_1$  indicating the activity coefficient of the sodium ion,

$$\log f_1 = -0.5 \pm 0.2 \quad \text{or} \quad \ln f_1 = -1.2$$

**Table 8.** (Calculated after McBain and Salmon).

c	t <sup>1</sup>	2.079t <sup>1</sup>	2.079t <sup>1</sup> - 2c	c <sup>3</sup>	K	K Mean	K <sup>1</sup>	K <sup>11</sup>
Potassium stearate.								
0.2	0.10	0.208	-0.192	0.0894	2.1			
0.5	0.17	0.353	-0.647	0.3536	1.8	1.8	5.4	2.3
0.75	0.19	0.395	-1.105	0.6495	1.7			
1.0	0.20	0.416	-1.584	1.0000	1.6			
Potassium palmitate.								
0.2	0.12	0.249	-0.151		1.7			
0.5	0.19	0.395	-0.605	do.	1.7	1.6	4.8	2.1
0.75	0.23	0.478	-1.022		1.6			
1.0	0.24	0.499	-1.501		1.5			
Potassium myristate.								
0.2	0.13	0.270	-0.130		1.5			
0.5	0.23	0.478	-0.522	do.	1.5	1.4	4.2	1.8
0.75	0.27	0.561	-0.939		1.4			
1.0	0.28	0.582	-1.418		1.4			
Potassium laurate.								
0.2	0.15	0.312	-0.088		1.0			
0.5	0.26	0.541	-0.459	do.	1.3	1.2	3.6	1.6
0.75	0.30	0.624	-0.876		1.3			
1.0	0.32	0.665	-1.335		1.3			
Potassium caprate.								
0.2	0.16	0.333	-0.067		0.7			
0.5	0.31	0.644	-0.356	do.	1.0	0.9	2.7	1.2
0.75	0.42	0.873	-0.627		1.0			
1.0	0.52	1.081	-0.919		0.9			
Sodium behenate.								
0.2	0.09	0.187	-0.213	do.	2.4	2.3	6.9	3.0
0.5	0.11	0.229	-0.771		2.2			
Sodium stearate.								
0.2	0.11	0.229	-0.171		1.9			
0.5	0.18	0.374	-0.626	do.	1.8	1.7	5.1	2.2
0.75	0.22	0.457	-1.043		1.6			
1.0	0.23	0.478	-1.522		1.5			
Sodium palmitate.								
0.2	0.13	0.270	-0.130		1.5			
0.5	0.20	0.416	-0.584	do.	1.6	1.5	4.5	1.95
0.75	0.24	0.499	-1.001		1.5			
1.0	0.25	0.520	-1.480		1.5			



where the uncertainty indicated answers to half the liquid junction potential itself.  $f_1$  is determined as before by  $a_1/c$  and  $a_1$  can be calculated from the potential difference between two sodium amalgam electrodes, one containing the soap solution and the other a sodium chloride solution of known activity (cf. (52) p. 15). This activity can be roughly determined when, as in Salmon's experiments, we are using two liquids for comparison of different concentrations, when we assume that the activity coefficient of the sodium ion in the liquid for comparison is determined by an equation of the form:

$$\log f = -k \sqrt[3]{c_{\text{NaCl}}}$$

The calculations thus give, for a 1n sodium palmitate solution:

$$\begin{aligned} \log f_1 + \log f_s &= -K^{11} = -1.95 \\ \log f_1 &= -0.5 & \log f_s &= -1.5 \\ f_1 &= 0.3 & f_s &= 0.03 \\ a_1 &= 0.3 & a_s &= 0.03 \end{aligned}$$

Reckoning here according to the classical dissociation theory, we obtain, like McBain and Salmon, — setting the activities found as equal to the true concentrations  $c_1^1$  and  $c_s^1$  —

$$c_1^1 = 0.3 \qquad c_s^1 = 0.03$$

as  $f_1^1$  and  $f_s^1$  must both be one (cf. p. 11) which means that 2/3 of the sodium ions and 97 % of the palmitate ions have disappeared and gone over into micellæ. The rise of boiling point, represented by  $c_1^1 + c_s^1$  is  $(0.3 + 0.03)/2.079 = 0.16^\circ$  while that found is  $0.25^\circ$ . The  $0.09^\circ$  should then be due to micellæ.

That such a calculation should be possible at all, and that it gives a positive value for the pressure of the micellæ (the rise of boiling point) shows that sodium palmitate does not behave abnormally in osmotic respects (see also *infra* p. 11).

## 2) General Theory.

The whole argument is however, subject to very considerable doubt. And the same applies to the ideas which Mc Bain associates with conductivity measurements in soap solutions, and which it is very difficult to estimate at their proper value. In the first place, there is great inaccuracy owing to the experimental error, and secondly, we know, from the modern work of

Bjerrum and Debye-Hückel, that it is necessary to take into consideration, for instance, the forces between the ionic charges and their influence on the activity of the ions; any calculation of true concentrations based on activity and osmotic measurements where these forces are disregarded must therefore be considered doubtful from the outset. Actually, we do not know what are the values of  $c_1^1$  and  $c_n^1$ , unless we know either  $f_1^1$  or  $f_n^1$  (and our purpose here of course is just to determine these) or can take them as equal to 1. And this we can only venture to do when the state of the ions is determined by the simple gas laws, which only hold good when the field of force in which an ion is situated is itself independent of the concentration of the surrounding ions. But that it should be possible to assume this at the outset, with such concentrated solutions as 1 n, seems to be very improbable.

Similarly, the conductivity of the soap solution is dependent upon interionic forces.

Since then, the calculation of the true concentrations of ions and micellæ in the solution encounters insuperable difficulties, and as we have seen in the previous section that there are cases where it is not possible at all even to arrive at a numerical agreement with experience by association calculations of this sort (without regard to the interionic forces) we will in the following assume that sodium palmitate is completely dissociated, even at concentrations up to 1 n, into monovalent sodium ions and palmitate ions, the concentration of which will thus be:

$$c_1^1 = c \qquad c_n^1 = c$$

It must be pointed out that there is no direct proof of this assumption; we will, however, allow it for the sake of simplicity, as we can then see more clearly what possibilities exist for an explanation of the low osmotic pressure by other means than micella hypothesis. Allowance can always be made for this later on should it prove needful.

We are thus faced with the necessity of explaining the position solely by assumptions as to the forces between the ions. As the concentrations are considerable, the foundation upon which Bjerrum's and Debye-Hückel's theory for dilute solutions is based will here be lacking, and although the forces between the ionic charges play a considerable part, they are never-

theless not sufficient to explain the difference between the state of a soap solution and for instance a NaCl solution, which also contains two monovalent ions. Unfortunately, the theory for concentrated electrolyte solutions is but very little developed. In the present case, I have not been able to apply the theories by N. Bjerrum<sup>1)</sup> and E. Hückel<sup>2)</sup> which have appeared of late years, and which do in various ways explain the conditions in concentrated solutions; I have therefore, as in a previous paper<sup>3)</sup> formed some qualitative ideas as to the importance of van d. Waals' cohesion forces for the interaction between the ions in an aqueous solution, where it is to be expected that we have to deal with forces very closely associated with the constitution of the ions.

In this previous work, I have endeavoured, on the basis of Debye's and Fajans' ideas as to mutual polarisation, deformation of the atomic systems of the molecules and the ions, with the importance of the same to the forces of cohesion, to explain certain regularities in the salting out of neutral substances with salts of different characters. I have here, to make the position clearer, pointed out some analogies between the gases and the solutions, using, for the former, the formulæ given by Debye<sup>4)</sup> for cohesion forces between quadrupoles, which serve to determine  $a$  in the following equation:

$$\left(p + \frac{a}{v^2}\right)v = RT \left(1 + \frac{b}{v}\right) \quad (67)$$

by the expression:

$$a = \frac{9}{10} P_0 N \cdot \frac{t^2}{d^5} \quad (68)$$

where  $P_0$  is the molecular refraction of the gas,  $N = 6.06 \cdot 10^{23}$ ,  $t$  the mean momentum of inertia of the electric charges in the molecule, and  $d$  the diameter. The volume correction is as we know determined by

$$b = \frac{2}{3} N \cdot \pi d^3 \quad (69)$$

On the basis of (67) we can obtain the following expression for the activity coefficient  $f$  of the gas

<sup>1)</sup> Zeitschr. f. anorg. Chem. **129**, 323 (1923).

<sup>2)</sup> Physik. Zeitschr. **26**, 93 (1925).

<sup>3)</sup> Compt.-rendus d. Lab. Carlsberg **15**, No. 4 (1923).

<sup>4)</sup> Physik. Zeitschrift **21**, 178 (1920).

$$\log f = 0.8686 \left( b - \frac{a}{RT} \right) c$$

where  $c = \frac{I}{V}$

and we see from this that a value of  $a/RT$  high in proportion to  $b$  will be capable of rendering  $\log f$  negative, and thus making  $f$  less than 1. Now  $a$  increases with increasing  $P_0$  and  $t$ , but decreasing  $d$ , whereas  $b$  decreases with decreasing  $d$ , so that a high polarisability combined with small volume may give rise to a highly attractive field of force between the molecules, and thus a small  $f$ . As the following calculation shows,  $a$  will, with increasing size of molecules, increase more than  $b$ , so that  $f$  decreases with increasing size of the molecules.

For hydrogen, where  $b$  is 16.6 cc and  $a$  is  $0.15 \cdot 10^{12}$  dyn cm<sup>4</sup>, we obtain:

$$\log f = +0.010 \cdot c \quad (\text{at } 100^\circ)$$

which for  $c = 1$  gives

$$f = 1.023.$$

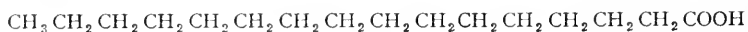
For pentane C<sub>5</sub>H<sub>12</sub>, where  $b$  is 145 cc and  $a$   $19.2 \cdot 10^{12}$  dyn cm<sup>4</sup>, we obtain:

$$\log f = -0.411 \cdot c \quad (\text{at } 100^\circ)$$

which for  $c = 1$  gives

$$f = 0.388$$

Even in the case of gases whose molecules are not quadrupoles, or whose molecules cannot be regarded as spherical, we can with certainty assume that the same effect as that above calculated will appear,  $f$  decreasing with increasing size of molecules. A gas with molecules of the same structure as palmitinic acid, whose formula we can, for the sake of illustration, write in full:



would, on account of the relatively slight volume of its molecules and their doubtless extremely high deformability, have a very slight activity. It would therefore seem natural to explain the behaviour of the soaps by cohesion forces of this sort, especially as the effect here is really of the same order of magnitude as for the gases, which is plainly evident from Table 9, where  $(b - a/RT) \cdot 0.8686$  is calculated for the gases from methane to octane ( $T = 373^\circ$ )

Table 9.

Substance	$a \cdot 10^{-12}$ dyn cm <sup>4</sup>	b cm <sup>3</sup>	a/RT cm <sup>3</sup>	0.8686 (b - a/RT) Liter
CH <sub>4</sub>	1.82	36.4	58.55	-0.019
C <sub>2</sub> H <sub>6</sub>	6.05	69.9	195.0	-0.117
C <sub>3</sub> H <sub>8</sub>	8.78	84.8	283.2	-0.172
C <sub>5</sub> H <sub>12</sub>	19.28	146.0	621.2	-0.413
C <sub>6</sub> H <sub>14</sub>	25.07	175.9	808.0	-0.554
C <sub>7</sub> H <sub>16</sub>	31.96	266	1030	-0.664
C <sub>8</sub> H <sub>18</sub>	37.85	237	1220	-0.854

bearing in mind that for the soaps,

$$\log f_s = -K^1 - \log f_1$$

for  $c=1$ , whereas for the gases,

$$\log f = 0.8686 (b - a/RT)$$

for  $c=1$ .

As  $\log f_1$  doubtless in the majority of cases lies somewhere about 0.3 — 0.5, we can, by comparison with Table 8, see the agreement between  $f$  and  $f_s^1$ ).

Certain difficulties however, here arise if we apply the same ideas of cohesion forces to the case of solutions; ideas based, as we see, on the mutual interaction between the dissolved molecules' electric fields (we may call them neutral fields for purposes of distinction from the field arising out of any possible ionic charge):

If, to any solvent D, we add a substance S, the constitution of whose molecules presents many points of similarity with that of the molecules of the solvent itself, it is highly probable that S will form a very nearly ideal mixture with D, which again means that the activity of S, throughout a large interval of concentration, will be proportional to the concentration indicated in mol. per 1000 g of the solvent. For the alteration of the activity coefficient with the concentration, due to alteration in the field of force in which any given molecule S is situated, will gradually, as the molecules of D, with increasing concentration, are replaced by the very similar molecules of S, become very slight.

<sup>1</sup>) Cf. eg.  $\log f = -0.854$  for octane ( $c=1$ )

and  $\log f_s = -1.2 + 0.3 = -0.9$  for the caprate ion ( $c=1$ ) for which the number of carbon atoms is 10.

As an instance of such a system, we might imagine sodium palmitate dissolved in melted palmitinic acid, as there can hardly be any doubt but that the Van d. Waals cohesion forces between the palmitate ions would here be without significance, leaving only the forces between the ionic charges and the neutral fields of the sodium ions.

It is important to point out, in this connection, that solutions of soaps in ethylalcohol  $\text{CH}_3\text{CH}_2\text{OH}$  do not exhibit the anomalies found in aqueous solutions.

Since then the cohesive action between the molecules of S in any given solvent — and therefore also in water — can be zero, there is a possibility of both positive and negative effect; i. e. substances whose molecules' affinity to water is greater than their affinity one to another, and which would therefore, at increasing concentration, show an increasing activity coefficient answering to a decreasing cohesion effect (e. g.  $\text{HCl}$ ,  $\text{LiCl}$ ) and substances whose affinity one to another is greater than their affinity to water and which therefore show a decreasing activity coefficient answering to an increasing cohesion action. Sodium palmitate should thus be an example of this.

In order to determine when we should look for one and when the other, the following rule may be given:

If the alteration of energy,  $U$ , on transference of a molecule of S from the liquid state to the dissolved state in D be negative, we may look for an activity coefficient decreasing with increasing concentration. If  $U$  be positive, the activity coefficient will increase with the concentration. As the activity coefficient may with advantage be taken as equal to 1 at infinite dilution, this means that  $f$  is greater than 1, in the first, and less than 1 in the second case.

Such transference affords indeed, information as to the alteration in the state of an S molecule when the surrounding S molecules are replaced by D molecules (cf. also K. Linderstrøm-Lang l. c. p. 2) which also occurs when a solution of S in D is diluted with D.

This rule applies roughly to several substances. In the case of the electrolytes, it is somewhat masked partly by the influence of the interionic forces from the ionic charges, partly also by the fact that we often have to deal with solids. According to

Table 10 however, we find, also here, the regularity that  $f$  or  $\varphi$  is often less the greater  $U$  is.

Table 10.

Substance	U	$\varphi(c=1)$	$f(c=1)$
HCl	+ 17.3	1.06	0.829
KOH	+ 12.5		0.786
LiCl	+ 8.4	1.02	0.752
HNO <sub>3</sub>	+ 7.5	1.01	
NaCl	- 1.0	0.90	
KCl	- 4.4	0.88	0.593
NaNO <sub>3</sub>	- 4.7	0.81	
KNO <sub>3</sub>	- 8.5	0.69	

It is therefore important to note that the heat of solution for sodium palmitate in water owing to the enormous increase of the solubility with the temperature, may be considered as having a very high negative value<sup>1)</sup>.

Apart from these indications, it must be pointed out that when we consider such a series of homologous substances with increasing molecular weight, such as the salts of the fatty acids, the variation in the state of their aqueous solutions with the length of the carbon chain will doubtless be qualitatively estimated by noting their varying volume and deformability from substance to substance, as there will otherwise be so many points of similarity between the links of the series that these qualities pointed out are just those which are most variable.

When therefore we consider McBain's and Salmon's curves for the alteration in rise of boiling point with the concentration, and with the nature of the soaps, or consider Table 8, where  $K$  and  $K^1$  are calculated, the regularity with which  $\varphi$  and  $f$ , owing to increasing  $K$  and  $K^1$ , decrease with increasing length of the negative ion's carbon chain, is very characteristic. We therefore consider it natural to attribute the slight osmotic pressure of soap solutions to the influence of an extremely powerful interaction between the fatty acid ions themselves. This also has the

<sup>1)</sup> A preliminary determination we have made seems to show that the heat of solution about 70° is  $< -35$  for sodium stearate.

effect of rendering  $f_s$  extremely small. In this interaction, the cohesion forces between the long and pliable carbonic chains play a dominant part.

The principal reason why we do not consider that the carbon chains unite to form micellæ is partly that it is not necessary in order to explain the results, and partly that several of the qualities of soap solutions are most simply explained by not assuming association. We shall return to this point later on (see p. 39).

### 3. *Stability of soap solutions.*

In considering the stability of soap solutions, we shall find equation 44 b p. 12 best suited to the purpose.

$$\left(\frac{\partial \varphi}{\partial c}\right)_{p,T} \geq 0 \quad \text{or according to (22)} \quad \left(\frac{\partial t^1}{\partial c}\right) \geq 0 \quad (71)$$

Looking at Fig. 2 it will be seen that instability occurs from  $C_{12}$  and upwards at concentrations about 1 n. This can also be approximately calculated from the equations (65)

$$\varphi \cdot 2c = 2c - Kc^{\frac{3}{2}}$$

whence

$$\frac{\partial \varphi}{\partial c} = 2 - \frac{3}{2} K \sqrt{c} \quad (72)$$

which at the limit of stability — let us call it  $c_K$  — gives

$$2 - \frac{3}{2} K \sqrt{c_K} = 0$$

$$c_K = \frac{16}{9K^2}$$

From this we obtain:

For potassium laurate..  $c_K = 1.3$

— myristate 0.9

— palmitate 0.7

In the case of stearic acid and behenic acid,  $K$  is not sufficiently constant to give any information.

And now, what about the condition

$$\frac{\partial \ln a_s}{\partial c} \geq 0 \quad (73)$$

— is this satisfied, or have we here the same thing as in the case of osmotically abnormal electrolytes, that

$$\frac{\partial \ln a_s}{\partial c} < 0 \quad (74)$$



For though the soaps do not behave abnormally in osmotic respects, this does not necessarily imply that (73) is satisfied. For whether the integral

$$\int_0^c \frac{da_s}{f_s}$$

is negative or not (see p. 21) merely depends on how much  $a_s$  has decreased with increasing  $c$  in the interval from 0 to  $c$ . It is possible that — within the limit of stability —  $\int_0^c \frac{da_s}{f_s}$  may be negative, but this integral need not be negative even though  $a_s$  may have decreased, or may decrease, with increasing  $c$ . For  $a_s$  can in the first part of the concentration interval from 0 to  $c_i$  — as we may call the concentration at which  $\frac{d \ln a_s}{d c}$  changes its sign from + to — — have increased to such a degree that the sum of the integral's two parts from 0 to  $c_i$  and from  $c_i$  to  $c$  will be positive.

Unfortunately, the experimental material does not permit of any certain determination whether the case indicated by (74) occurs or not. We will nevertheless make the following assumption, pointing out at the same time its approximative character.

We have found (p. 25) for the activity coefficient of the sodium ions

$$\ln f_1 = -1.2.$$

We now assume that  $f_1$  is a function of the same form as  $f_1 \cdot f_s$  or bearing in mind (64)

$$\ln f_1 = -1.2 \sqrt{c} \quad (75)$$

This gives us:

$$\ln f_s = (-K^1 + 1.2) \sqrt{c} \quad (76)$$

whence, as

$$\begin{aligned} \ln a_s &= \ln c + \ln f_s \\ \frac{\partial \ln a_s}{\partial c} &= \frac{1}{c} - \frac{K^1 - 1.2}{2} \frac{1}{\sqrt{c}} \end{aligned} \quad (77)$$

$c_i$  (cf. supra) is therefore determined by the equation:

$$\begin{aligned} \frac{1}{c_i} - \frac{K^1 - 1.2}{2} \frac{1}{\sqrt{c_i}} &= 0 \\ c_i &= \left( \frac{2}{K^1 - 1.2} \right)^2 \end{aligned} \quad (78)$$

From this we obtain, for sodium palmitate (see Table 8)

$$c_i = \left( \frac{2}{3.3} \right)^2 = 0.37 \text{ n} \quad (79)$$

At this concentration, then  $a_s$  should begin to decrease with increasing concentration. We see that this concentration is less than half that at which the solution becomes really unstable.

Looking at Fig. 2, we can, it seems to me, hardly help noticing the remarkable resemblance between these curves and those obtained by calculating the relation between pressure and volume of a non-ideal gas according to Van d. Waals' equation (cf. Fig. 1). For the lower items of the soaps up to the laurates, the course of the curves lies as for the gases above the critical temperature (cf. Table 9). At the higher stages, which, owing to the larger ions, must be assumed to correspond to gases with high critical temperature, the course of the curves lies through a maximum and a minimum, and it might seem as if we had here an instance of something corresponding to that for gases unrealisable part, which normally runs in a straight line owing to liquid formation. That portion of the curve between the maximum and the minimum answers, as we have seen, to absolutely unstable conditions, and the fact that the system does not apparently divide into two phases may be due to slight rate of reaction e. g. to the fact that the one phase is continuously dispersed throughout the other: A gel is formed.

The section of the curve on the left of the minimum however, can perhaps be assumed to answer to stable conditions, as here again

$$\left( \frac{\partial \varphi}{\partial c} \right) > 0$$

According to Van d. Waals' equation the ascending branch of the curve is due to a dominant volume effect, and it is in reality not strange that we cannot add unlimited quantities of soap to the phase and still find abnormally low pressures.

In detail, the conditions are naturally very different from those of the gases. The expression for activity coefficient obtainable from Van d. Waals' equation, is as mentioned

$$\log f = k \cdot c$$

whereas the formula

$$\log f = k_1 \sqrt{c}$$

will suffice well enough for most of McBain's experiments. Considering the influence which must be exerted by the electric charges of the ions, this difference is natural enough. Both formulæ are only valid for low concentrations, and not at the above mentioned critical part. Here, as we know, the theoretical foundation of Van d. Waals' equation fails us, even for gases, and it must therefore, at the present stage, be regarded as still more impossible with a model for the palmitate ion such as that here assumed, to give any satisfactory theoretical explanation of the peculiar shape of the curves. It is also, owing to the great experimental error involved in these experiments, difficult to calculate suitable empirical expressions with advantage.

The experiments in question were carried out at  $90^{\circ}$ . It would be most interesting if we could investigate the conditions especially at lower temperature. Here however we are faced with the difficulty that many of the soaps are slightly soluble; in other words that their solutions are, at lower concentrations, practically unstable with regard to discontinuous formation of a new phase, that of soap in solid form. With the oleates however, which are soluble at ordinary temperature, a thorough investigation of the osmotic pressure and activity of the sodium ion should be practicable at a considerable range of temperature.

We should expect to find, at lower temperatures, far more abnormal conditions than at  $90^{\circ}$ . We see for instance from (70) that the term  $a/RT$ , at falling temperature becomes more and more dominant, so that  $f$ , and in the case of the soaps  $f_s$  must decrease in proportion to this. Possibly sodium oleate may be osmotically abnormal at ordinary temperature.

The melting and coagulation of gels is doubtless to some extent dependent on the relation of  $f_s$  to the temperature.

#### 4. *Internal physical state of soap solutions.*

Let us consider a 1 n sodium palmitate solution, where  $a_1 = 0.3$   $a_s = 0.03$ . In this solution, the mean distance between the sodium ions is abt.  $10 \cdot 10^{-8}$  cm, while the palmitate ions are abt.  $25 \cdot 10^{-8}$  cm long, or more than twice as large. These large ions can, according to our assumption, not be regarded as stiff, but must, under the influence of the thermic movement, affected by the electric fields of the water molecules, with those of the sodium ions, and above all their own mutual field of force, be undergoing constant de-

formation; they must be in ceaseless movement, affecting one another with enormous force, tangled up in one another like the threads in cotton-waste, so that it is hardly possible at any point to distinguish between different groups and say: this is a micella, here we have a single ion, that is a neutral colloid, these particles are subject to the gas laws. At one end of the ionic chain is the electric charge, and between these ends of the ions there must therefore be repulsion. It is, we hope, apparent from the foregoing that it is just these charges which determine the stability.

Between these chains, the sodium ions, owing to their small size, move about like the electrons in a metal affected by the electric forces, not associated and yet not free — just as in an ordinary electrolyte solution, though we are here dealing with more intensive electric fields, so that  $f_1$  is also smaller than for instance in a sodium chloride solution of the same concentration. It seems obvious that the size of the sodium ions must play a great part in the constitution of the solution. The strength of the »screening« effect which they must occasion by entering between two soap ions with the same charge must depend on the minimum distance between the soap ions at which this is possible at all. For the cohesive force between two molecules decreases very strongly, when the distance between them increases (cf. Debye l. c.). If therefore the sodium ions are replaced by large positive ions (see Table I) with pronounced central symmetry and thus presumably of large volume, it is possible that the diminished repulsion between two negatively charged soap ions occasioned by the appearance of the positive ions between them would be of no importance to the magnitude of the cohesive force, as this is already insignificant at such distances between the soap ions where any entry of large positive ions can take place. Consequently the forces of attraction between the soap ions must decrease with increasing volume of the positive ion, and both  $f_s$ ,  $f_1$  and  $\varphi$  will increase. This is precisely indicated in the Hammarsten effect. We shall presently see that this explanation can in reality be imagined to coincide with Hammarsten's own.

### 5. *Concluding remarks.*

The conception here indicated seems to me to present considerable advantages. We cannot disregard the existence of these forces, and the constitution of the soap solution easily explains other phenomena. It is impossible in a single publication to deal with all sides of the question, and we will therefore merely draw attention to the following points:

The high viscosity of the soap solutions in particular seems to us very simply evident from the picture we have given in the previous section. We have often found it difficult to understand the justification of the hydration hypothesis most frequently employed for the explanation of the high viscosity of colloid solutions, as the enormous hydration values thus arrived at are hard to conceive. When we consider that the molecules of fatty acids show an aversion for water in the truest sense, being always forced out by the water molecules into the surface of the phase, and that the soaps reduce the surface tension of water and are therefore found in the highest concentration at the surface too, and finally, that the vapour tension of a soap solution lies very near the vapour tension of pure water, ( $t'$  being small,) it is not clear how an enormously powerful hydration of the soap ions could really take place.

The transition from sol to gel also seems to me to be simply and easily explained by the theory.

The most difficult point to explain, no doubt, will be the results of the conductivity determinations (McBain l. c.). This however, is really a field which, despite a very great amount of experimental material, has not yet been mastered to anything approaching the same degree as the states of equilibrium where thermodynamic methods can be employed.

The curve for relation between reciprocal equivalent conductivity and concentration run, for the soaps from the laurates upwards, through a maximum and minimum just as the curves for relation between  $t'$  and  $c$ , but the maximum, answering to a minimum of conductivity, lies right down at the concentration 0.1 to 0.2  $n$ , while that for  $t'$  lies about  $c = 1 n$ . McBain explains this by assuming that in the neighbourhood of the concentration 0.1  $n$ , rapidly moving micellæ take over the electric conduction. We consider that an explanation agreeing with this

in reality could be based on the idea of interionic forces, and we cannot regard it as mere accident, that the concentration at which conductivity begins to increase should lie not so far from  $c_i$  (0.37), the concentration where  $\frac{\partial \ln a_s}{\partial c}$  changes its sign, and which we have very roughly calculated already (see p. 35). At the moment however, for theoretical reasons, and owing to the great inaccuracy of the experiments, it is impossible to say anything definite.

We are well aware that the theory here advanced cannot as yet claim to be self-evident. But on the other hand, the presence of this enormous number of micellæ in soap solutions has never been proved, and can, from the foregoing, hardly be proved by osmotic experiment. The solutions of sodium stearate which we have investigated in various ways, as will be described in a later work, are, up to 0.8 at 90°, absolutely as clear as water, and show no trace of tyndall effect. The conception of micellæ as particles following the gas laws encounters manifold difficulties, and the calculation of the concentration of the different complexes on the basis of conductivity requires assumptions which at present have no sure foundation.

Nevertheless, we cannot disregard the possibility that there may exist association products; the forces we have assumed might very well be supposed to occasion the formation of such. These must however, apparently be in so close interaction with the remaining particles in the solution, that a determination of their concentration at the moment is impossible. It is therefore simplest and perhaps nearest to the truth to assume that no association takes place. If we are to reckon with any such, it must be taken to be of a similar type to that which, as Bjerrum (l. c.) has shown, is doubtless found even in a sodium chloride solution, and which is essentially different from that assumed by the classical dissociation theory. Complications of this sort would of course largely serve to render the functions by which  $f_s$  depends on  $c$  more complex, a point which we shall briefly discuss in the next chapter.

### E. Some Calculations Based on Hammarsten's Experiments. Conclusion.

We shall in the following make some calculations based on the principle given in Section A. We cannot sufficiently emphasise the fact that these are, owing to the inadequacy of the experimental material, and our complete ignorance as to what function  $f_{\text{H}}$ , the activity coefficient of the great ion, is of the electrolyte concentration, necessarily of a rough qualitative character only. We have however, made them in order to illustrate the applicability of the principle for future investigations, and because they are distinctly associated with the theory outlined in the foregoing. Take protamin chloride for instance. This is stated as having a molecular weight of 800, whereas that of palmitic acid is 256; and we might therefore reasonably expect to find here even more pronounced osmotic abnormality. And the calculations in Section C also show that this is the case. At far lower concentrations than for the sodium palmitate solutions, we find a marked reduction in the osmotic pressure, and, as far as we can see, an enormous reduction in the activity of  $I_{\text{H}}$ , the large ion. That the lower temperature at which Hammarsten's experiment was carried out is of decisive importance here can hardly be doubted.

#### 1. Protamin chloride.

In the following Table 11 the values found by H. Hammarsten (l. c. Table XXXIII p. 533) for concentration of the free chlorine ions in a protamin chloride solution are re-calculated to chlorine activities  $a_{\text{Cl}}$  after the formula p. 17.  $c_{\text{Cl}}$  is the total concentration of the chlorine ions in the solution.

$-\log f_{\text{Cl}}$  is equal to  $\text{pa}_{\text{H}} + \log c$  and  $-\log f_{\text{Cl}}$  (calculated) is found from the following empirical formula:

$$-\log f_{\text{Cl}} = 0.99 \sqrt[3]{c_{\text{Cl}}} - 2.46 c_{\text{Cl}}. \quad (80)$$

According to (80), we have

$$\int_0^{c_{\text{Cl}}} c_{\text{Cl}} d \ln f_{\text{Cl}} = -0.570 c_{\text{Cl}}^{\frac{4}{3}} + 2.83 c_{\text{Cl}}^2 \quad (81)$$

whence

$$\int_0^{c_{\text{Cl}}} c_{\text{Cl}} d \ln a_{\text{Cl}} = \int_0^{c_{\text{Cl}}} \frac{c_{\text{Cl}} da_{\text{Cl}}}{f_{\text{Cl}}} = c_{\text{Cl}} (1 - 0.570 \sqrt[3]{c_{\text{Cl}}} + 2.83 c_{\text{Cl}}) \quad (82)$$

Table 11.

$c_{Cl} \cdot 10^3$	$-\log c_{Cl}$	$a_{Cl} \cdot 10^3$	$pa_{Cl}$	$-\log f_{Cl}$	$\sqrt[3]{c_{Cl}}$	$-\log f_{Cl}$ (calculated)
42.3	1.374	24.2	1.616	0.242	0.348	(0.242)
32.8	1.484	19.5	1.709	0.225	0.320	0.239
16.4	1.785	10.2	1.992	0.207	0.254	0.212
8.56	2.067	5.66	2.247	0.180	0.205	0.183
4.28	2.369	3.06	2.515	0.146	0.162	0.150
2.14	2.670	1.62	2.792	0.122	0.129	(0.122)

If we assume that protamin is a single substance, we can from (18) and (22) obtain:

$$\int_0^{c_n} c_n d\ln a_n = \int_0^{c_n} \frac{da_n}{f_n} = \varphi (c_{Cl} + c_n) - \int_0^{c_{Cl}} \frac{da_{Cl}}{f_{Cl}} \quad (83)$$

and

$$\varphi (c_{Cl} + c_n) = 0.5380 t = 0.4038 \cdot 10^{-4} P. \quad (84)$$

The quantities

$$F_{Cl} = \int_0^{c_{Cl}} c_{Cl} d\ln a_{Cl} \text{ according to (82)}$$

and

$$F_n = \int_0^{c_n} c_n d\ln a_n \quad - \quad (83)$$

are noted in Table 12, calculated from H. Hammarsten's measurements of both freezing point lowering and osmotic pressure of protamin chloride solutions (l. c. Tables XXXIV and XXXVa pp. 533 and 534). Cf. also the present paper p. 18, Tables 2 and 3.

Table 12.

$c_{Cl} \cdot 10^3$	$a_{Cl} \cdot 10^3$	$\varphi \sum c_i$	$F_{Cl} \cdot 10^3$	$F_n \cdot 10^3$	$F_n/c_{Cl}$	$F_i/c_{Cl}^2$
42.3	24.2	25.8	39.0	- 13.2	- 0.31	- 7
29.7	17.5	18.8	25.8	- 7.0	- 0.24	- 8
21.2	12.8	12.9	19.1	- 6.2	- 0.29	- 14
14.6	8.77	8.99	13.2	- 4.21	- 0.29	- 20
7.31	4.82	4.75	6.65	- 1.90	- 0.26	- 35
3.62	2.46	2.38	3.37	- 0.99	- 0.27	- 74

From this it appears, in the first place, that  $\int_0^{c_{Cl}} \frac{da_{Cl}}{f_{Cl}}$  is



throughout much greater than  $a_{Cl}$  (cf. p. 21). Further, it seems as if  $F_n/c_{Cl}$  in the concentration interval is practically constant  $= -0.27$ . This gives:

$$F_n = \int_0^{c_n} c_n d \ln a_n = -0.27 c_n \cdot n, \text{ as } c_{Cl} = n c_n \quad (85)$$

or

$$c_n d \ln a_n = -0.27 \cdot n d c_n \quad (86)$$

This equation does not permit a determination of  $a_n$  as it makes  $a_n = \infty$  when  $c_n = 0$ . (86) cannot therefore be satisfied at lower concentrations. We can nevertheless perceive from the negative value of  $F_n$  that  $a_n$  and thus  $f_n$  must be quite extraordinarily small.

(86) does on the other hand permit a determination of  $\frac{\partial \ln a_n}{\partial c_n}$  as

$$\frac{\partial \ln a_n}{\partial c_n} = \frac{F_n \cdot n^2}{c_{Cl}^2} \quad (87)$$

It will be seen from the last column in Table 12 that  $\frac{\partial \ln a_n}{\partial c_n}$  the concentration interval in question, is of very high negative value, but that at higher concentration it is considerably less negative than at lower concentration. It is also apparent from (87) that this differential quotient depends to a very great extent on what value we ascribe to  $n$ , the valency of the protamin ions; it becomes more and more negative as  $n$  increases.

It is evident that the solutions are stable in regard to continued variation of phase, as

$$\frac{\partial \varphi \sum c_i}{\partial c_{Cl}} > 0 \quad \begin{array}{l} \text{(cf. (44 b) p. 12} \\ \text{and Table 12)} \end{array}$$

If protamin is a mixture of several substances, then  $\int_{f_n}^{\frac{da_n}{f_n}}$  must be replaced by  $\sum \int_{f_i}^{\frac{da_i}{f_i}}$  where the summation must be extended to all its independent components.

Owing to experimental difficulties, and as Table 12 comprises two series of experiments, of which one was made at  $0^\circ$  and the other at  $20^\circ$ , we must be content with these indications.

## 2. Conclusion.

Passing now from consideration of the soap solutions, and the approximated calculations in the foregoing section, to the theoretical examination of the state of such solutions as the Brothers Hammarsten have worked with, it must be pointed out that it will doubtless be difficult to maintain completely a picture such as that given in the case of the soap solutions. Although the  $f$  function must still be said to be little known, it is nevertheless evident that  $f_n$  is not a function of the same sort as in the soap solutions. It is therefore possible that we have here to deal with micella formation in a higher degree. Unfortunately, nothing definite can be said on this point at the moment. We regard it as the main result of our work have demonstrated the possible cause of osmotically abnormal behaviour of electrolytes, a feature which is altogether inexplicable by the classical dissociation theory. And we would in conclusion merely emphasise the following points:

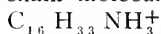
Throughout all our calculations we have reckoned with the activity coefficient  $f_i$  defined by the equation.

$$a_i = c_i \cdot f_i$$

where  $c_i$  is the total concentration of the ion marked  $i$ .  $f_i$  has been designated by Brønsted (l. c.) as the stoichiometrical activity coefficient, and is naturally a purely thermodynamic quantity. That it is small can (cf. the example p. 13) denote that an essential quantity of the ion to which it refers has passed over into micellæ, but the calculation of the concentration of these is, for osmotically abnormal electrolytes, attended by insuperable difficulties, which render it hardly worth while even where it can be done. We have therefore kept to this thermodynamic method. The correctness of this is limited only by the case where there is no equilibrium. If for instance some chlorine ions in the protamin chloride solution are so bound by the protamin ions that they are not in equilibrium with the free chlorine ions, then  $f_{Cl}$  will have no thermodynamic meaning, nor indeed, any other. The ease with which, by dialysis against salt solutions, the diffusible ion in these colloid salts can be replaced by another, convinces us, however, that such is not the case. The Hammarsten effect convinces us of another reversibility, to wit the reversibility of the micellæ formation itself. For if we deny the existence

of the cohesive forces, then there must, in solutions of the colloid salts with small ions, be an extensive association to micellæ, otherwise the osmotic results could not be explained at all. Now the micellæ contain a portion of the sodium ions, and it is doubtless their »screening« effect which prevents the micellæ from being exploded by too heavy a charge. The large ions which Hammarsten has combined with his colloid ions are not capable of taking part in the micella formation, and it is probable that this takes place to a far less extent. Hence the increased osmotic pressure, etc. On comparing with p. 38 we see that this explanation, which is very near to that of Hammarsten, actually agrees with the one previously given.

It is interesting to note in this connection that the large ammonium ions (Table 1) also behave normally in other salts and indeed, with increasing size exhibit an increased reduction of vapour tension in aqueous solution. This is doubtless due to the fact that the substituted aliphatic groups arrange themselves in point of space about the nitrogen atom, so that the volume in proportion to deformability (cf. p. 30) will here be far greater than in the case of a chain molecule. The positive ion



behaves, according to Krafft,<sup>1)</sup> exactly as do the soaps<sup>2)</sup>.

The assumption of such an ionic network as that described in Section D, 4 has, however, certain advantages in this connection. The transition from sol to gel becomes natural, and the viscosity conditions are easily and naturally explained. These organic ions are doubtless of an extremely complex structure, in most cases of the chain type, and are therefore well suited to fit into the picture. But the ionic net can hardly be regarded as continuous. There can be certain groups of closely linked ions which for a certain period follow the same course and through which we must, with Hammarsten, assume that the smaller ions can move. But the main thing is, that we have to assume that these groups are not finite systems, but interact with other groups through neutral fields.

It would be interesting, with a greater amount of experimental material, partially to relinquish the purely thermodynamic method and proceed to an examination of the position by means of equations such as (26), (27) and (28). There is reason to expect

<sup>1)</sup> Ber. d. deutsch. chem. Gesellsch. **29**, 1331 (1896).

<sup>2)</sup> See also M. H. Norris: Journ. Chem. Soc. **121**, 2126, (1922).

that a so marked dependence on the concentration as that exhibited by  $a_n$  in protamin chloride, may be partly eliminated by using a complex such as  $I_n, xCl^-$  as an independent variable. For we must have

$$a_{\text{compl.}} = k a_n \cdot a_{Cl}^x$$

whence

$$\frac{\partial \ln a_{\text{compl.}}}{\partial c_n} = \frac{\partial \ln a_n}{\partial c_n} + x \frac{\partial \ln a_{Cl}}{\partial c_n}$$

which shows that  $\frac{\partial \ln a_{\text{compl.}}}{\partial c_n}$  must be less negative than  $\frac{\partial \ln a_n}{\partial c_n}$  as  $\frac{\partial \ln a_{Cl}}{\partial c_n}$  is positive. The osmotic and activity experiments limit

the choice of  $x$ ; a question into which we shall not enter here.

We hope to revert to these questions in a later communication.

### Summary.

1. The general thermodynamic treatment of solutions is briefly described, with special reference to its application with colloid electrolyte solutions. On the basis of Gibbs' conditional equation for stability of a phase it is shown that it is possible for the potential (or activity) of one of the ions in an electrolyte solution within the stability limit to be a diminishing function of the concentration, as long as the potential of the electrolyte as a whole is an increasing function of the concentration.

2. On the basis of the important researches made by the Brothers Hammarsten with colloid electrolytes, consisting of a large ion  $I_n$  and a small ion  $I_1$  (chlorine ion etc.) in agreement with these writers, it is shown that the classical dissociation theory cannot apply to so-called osmotically abnormal electrolytes, i. e. those whose osmotically effective concentration is less than the activity of one of their ions. ( $I_1$ ).

3. It is shown that if we wish to deal with solution of osmotically abnormal electrolytes as monophase systems in internal equilibrium, then the only possible explanation of the results arrived at is (see 2) that the activity of the large ion  $I_n$  must be a diminishing function of the concentration. It is shown that only an extremely strong interaction between the large ions — possibly micella formation, only not in the classical sense — can give rise to this.

4. From the researches of McBain and his collaborators with soap solutions, a theory is formulated for the interaction between chain molecules, and it is indicated as probable that the cohesive forces between the chain ions present in the soap solutions are able to occasion the slight osmotic pressure, the high viscosity, gel formation, etc. We may assume that the soaps in aqueous solution are, at  $90^{\circ}$ , for a considerable concentration interval, molecular disperse.

An explanation of the Hammarsten effect is given.

5. With a view to illustrating the applicability of the thermodynamic method, some approximative calculations are made from H. Hammarsten's experiments with protamin chloride.

*Carlsberg Laboratory, April 1926.*

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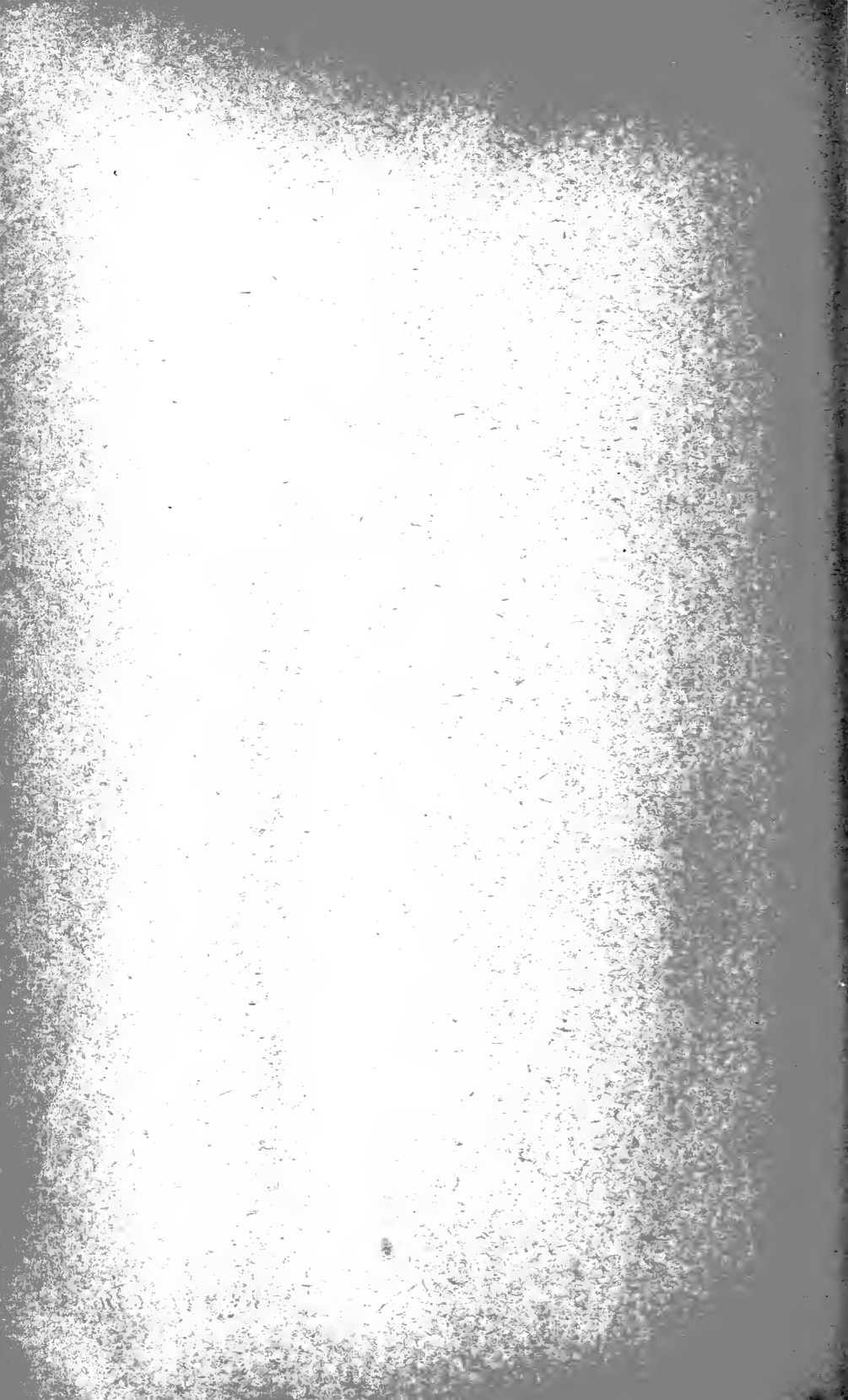
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# ON THE TWO COMPONENTS OF MALT DIASTASE.

BY

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That malt diastase is not a single enzyme, but a mixture of two distinct enzymes, is a theory which has long been generally adopted, though it has not been possible to separate the two components one from the other. The theory is based on certain investigations as to the influence of temperature on the decomposition of starch through the action of malt diastase.

Schwarzer<sup>1)</sup> investigated in 1870 the quantity of sugar formed when the starch has, through the action of the malt diastase, become just so far decomposed as to be no longer amenable to staining with iodine. He found that this quantity is lower at temperatures above 60° than at temperatures below; and also discovered that even at the lower temperatures, the formation of sugar is still but slight when the malt solution has previously been heated for some time to 70°. Schwarzer's results were soon confirmed by several other writers, notably O'Sullivan<sup>2)</sup> and Kjeldahl<sup>3)</sup> whose researches are of fundamental importance to the study of malt diastase.

Grützner<sup>4)</sup> is often<sup>5)</sup> credited with the discovery that the sugar-forming capacity of diastase can be impaired by heating. This, however, is not correct. He writes (l. c. p. 297):

»Wenn hiernach von einer bestimmten Menge Ptyalin in der Wärme und von derselben Menge Ptyalin gleich viel Stärke-

<sup>1)</sup> Schwarzer, Journ. f. prakt. Chemie. [2] **1**, 212 (1870).

<sup>2)</sup> O'Sullivan, Journ. of Chem. Soc. 1876, 125.

<sup>3)</sup> Kjeldahl, Comptes-rendus du Lab. Carlsberg **1**, 109 (1879).

<sup>4)</sup> Grützner, Pflügers Archiv **12**, 285 (1876).

<sup>5)</sup> E. g. Oppenheimer, Die Fermente, Bd. I, 679 (1925). Hizume, Biochem. Zeitschr. **146**, 52 (1924), m. fl.

kleister in der Kälte zersetzt wird, so ergibt sich, dass — natürlich innerhalb gewisser Zeit — nur die letztere Portion Erythrodextrin enthält, während die erstere, in welcher das Ptyalin durch die Kraft der Wärme unterstützt wurde, kein Erythrodextrin, wohl aber Zucker in grossen Mengen aufweist.«

Grützner, then, has not considered in the least what would be the effect of previous heating of the enzyme solution: he has simply investigated the hydrolysis of starch at different temperatures, with qualitative samples. He does not even say what were the temperatures in question, but from his other descriptions, it would seem as if the experiments with heat had been carried out at 37°, and those with cold at ordinary indoor temperature. His result, that the higher temperature gives more sugar than the lower, would then only imply that the enzyme is more powerfully active at 37° than at indoor temperature. Grützner's work is thus of very slight importance, and not deserving of further mention in this connection.

Bourquelot<sup>1)</sup> in a smaller work frequently quoted, has investigated the alteration occasioned in malt diastase by heating, and was the first to formulate the hypothesis that the diastase consists of a mixture of two or more distinct enzymes, which are successively destroyed as the temperature increases. Some months later, however, Bourquelot<sup>2)</sup> published a more comprehensive work on the same subject. This important work, in which he again emphasises the probability of the two-enzyme theory, seems now almost forgotten, and is hardly to be found quoted anywhere.

During recent years, several writers have investigated both the sugar-forming and the starch-decomposing capacity of malt diastase, in their relation to various chemical and physical factors. We shall not here give any account of the result of these researches, but refer the reader to the digests and lists of works in the current handbooks<sup>3)</sup>. Particularly noteworthy are, first and foremost, the comprehensive works of Euler and his pupils, those of Sherman and others, of Samec, van Laer, and the somewhat older works of Fernbach, Maquenne and Roux, etc.

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<sup>1)</sup> Comptes rend. de l'Acad. des Sciences **104**, 576 (1887).

<sup>2)</sup> Annales de l'Institut Pasteur **1**, 337 (1887).

<sup>3)</sup> Oppenheimer, Die Fermente, I, (1925). Euler, Chemie der Enzyme, II, 1, (1922).

Attempts to separate the two enzymes one from the other have however, been largely fruitless. The selective adsorption method, with which Willstätter and his collaborators attained such magnificent results, do not appear to have been employed for division of diastase itself into its component parts, but only for separating diastase from other enzymes. It would of course be most valuable if the method could be applied to such cases as that here in question.

The investigations described in the following pages<sup>1)</sup> were commenced, and to a very great extent carried out, at the Carlsberg Laboratory in Copenhagen. They were afterwards continued at the chemical and medico-chemical institutions at the University of Lund.

I am glad to take this opportunity of thanking Professor S. P. L. Sørensen, M. D., Ph. D., head of the Chemical Department of the Carlsberg Laboratory, for the years spent under his guidance at the Laboratory (where I was working as a guest), for the important tasks entrusted to me, and for the interest which he has always evinced in my work.

I have also to thank Professor E. M. P. Widmark, M. D., Head of the Medico-chemical Department at Lund, for his kindness in according me facilities for work at that institution.

## 1. Names of the Enzymes.

The investigations described in the following show that malt diastase — in accordance with Bourquelot's hypothesis — consists of two separate enzymes. In contrast to what was previously expected however, the reactions determined by these enzymes do not proceed successively, but parallel one to the other. Malt diastase contains, not an *amylase* decomposing starch to dextrans, and a *dextrinase* decomposing these dextrans to maltose, but two separate amylases, each capable of affecting the starch. The reaction produced by the one amylase gives reaction-products among which the dextrans predominate, while the reaction produced by the other amylase gives reaction-products among which maltose predominates. In accordance with this, the first amylase is called *dextrinogenamylase* and the other *saccha-*

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<sup>1)</sup> A brief account has already been given in C. r. de la Soc. de Biol. **87**, 1183 (1922).

*rogenamylase*. In a preliminary statement<sup>1)</sup> I used the shorter terms dextrinogenase and saccharogenase; these however, are inadequate inasmuch as they do not, as is now customary, contain the name of the substrate, but only of the reaction products. The longer names, giving both substrate and reaction product, seem to me preferable.

## 2. Methods of Determination.

a. The activity of dextrinogenamylase is determined according to Wohlgemuth<sup>2)</sup> as follows: different quantities of the enzyme solution in question are placed in test tubes and diluted with water to a volume of 1 cc. Then 1 cc of buffer solution and 1 cc of 0.2% solution of soluble starch are added, after which the test tubes are placed in a water thermostat at 38°.

For the buffer, phosphate is used; unless otherwise stated, a  $\frac{1}{15}$  molar phosphate mixture of the composition «0.12 secondary» according to Sørensen. This phosphate mixture has itself a hydrogen ion concentration of  $p_H = 5.0$ , but the buffer action of the phosphate at this concentration of hydrogen ions is so slight that the complete reaction solution has a hydrogen ion concentration of  $p_H = 5.4$ .

The test tubes are left standing in the thermostat for 30 minutes, and then cooled with cold water. To each tube is added one drop of abt.  $\frac{n}{50}$  iodine solution, and the colour is noted. In the first tubes, containing the largest quantities of enzyme, the colour will be an extremely pale yellow, almost colourless, which term will be employed in the following pages. In the next tubes, the colour is violet or brown, and in the last ones, where there is least enzyme, it is blue. We thus obtain two very clear and distinct boundary lines; one between colourless and violet, and one between violet and pure blue. The latter is sometimes a little difficult to determine; as to the former, however, it is only in exceptional cases that there is any possibility of doubt as to where the boundary should be drawn.

The quantity of enzyme in the last colourless tube is greater than is needed completely to hydrolyse the starch to substances

<sup>1)</sup> C. r. de la Soc. de Biol. **87**, 1183 (1922).

<sup>2)</sup> Wohlgemuth, Grundriss der Fermentmethoden. Berlin 1913. P. 43, and Biochem. Zeitsch. **9**, 1 (1908).

not stained by iodine. The quantity of enzyme in the first violet tube is not sufficient to produce this effect. Starting from the last colourless tube, Wohlgemuth calculates the expression  $2/v$ , which he uses as a measure of the activity of the enzyme solution.  $v$  denotes the number of cc enzyme solution contained in this tube, while the figure 2 indicates that the tube contains 1 cc 0.2 % starch solution.

This method of calculation is entirely satisfactory as long as the quantity of enzyme is always varied in the same way throughout all series of experiments. But as it was found advisable in these investigations to vary the quantity of enzyme in the tubes in more than one way, I have slightly modified Wohlgemuth's method of calculation, the value  $2/v$  being reckoned for both the tubes between which the boundary lies. The mean of these two figures is then used as a measure of the activity of the enzyme solution, and is indicated in the following pages by X.

Similarly, starting from the boundary line between the last of the violet tubes and the first of the pure blue, we can calculate another expression for the activity of the enzyme, indicated in the following by Y.

In accordance with Wohlgemuth's method, the quantities of enzyme in the different tubes in an experiment constitute a geometrical series. I have made use of two different series. In one, noted as »Series A«, the amount of enzyme in one tube is twice that in the next. In the other series, »Series B«, the amount of enzyme in one tube is 1.29 times that in the next. There are practical difficulties in the way of further reducing this proportion, and thus increasing the accuracy of the measurements.

Series A is obtained by progressive dilution of the enzyme solution after Wohlgemuth. In Series B, the enzyme is diluted in the proportion of 1:25, 1:50 or 1:100, and from the solutions thus obtained, the requisite quantities can be measured off with a graduated pipette. Table I shows the quantities of enzyme in each tube in both series. The values for activity, calculated from the position of the boundary lines, are given in Table 2.

Here and there in the following will be found what is apparently an accidental increase in the activity of an enzyme, where we should have expected to find a decrease. This is not due to experimental error, but solely to the method of calculation; the error arises when passing from one series to another.

Table 1.

Tube	Series A	Series B	Boundary between tubes	Series A	Series B		
					1 : 25	1 : 50	1 : 100
1	1	1.00	0—1	0	—	—	—
2	0.5	0.77	1—2	3	57	115	230
3	0.25	0.60	2—3	6	74	148	296
4	0.125	0.46	3—4	12	96	192	384
5	0.062	0.36	4—5	24	124	247	495
6	0.031	0.28	5—6	48	159	317	635
7	0.016	0.22	6—7	100	203	406	812
8	0.008	0.17	7—8	190	261	521	1040
9	0.004	0.13	8—9	380	340	679	1360
10	0.002	0.10	9—10	770	442	885	1770
11	0.001	0.08	10—11	1500	563	1130	2250
12	0.0005	0.06	11—12	3100	729	1460	2920

Take for instance Exp. I: 9, p. 13. X first falls from 384 to 230, rising again, however, to 247. From Table 2, it will be seen that the activity  $X = 230$  is reached at a dilution of 1 : 100, and that the boundary then lay between tubes 1 and 2. We might then imagine that the boundary next time would lie below tube 1, and the experiment thus far prove fruitless. In order to avoid this, the next determination was made with a dilution of 1 : 50 only, which gave the value 247. According to Table 2, the boundary then lies between tubes 4 and 5. But if we calculate the value for the boundaries instead of the mean value, we obtain, in the first instance, X between 200 and 260, and in the second, X between 217 and 278. There is thus nothing to suggest that the value has not really suffered a decrease. If then, we are to draw any conclusions from an alteration in the values of X and Y, the alteration must not be too slight, especially in cases where we have to pass from one series to another. With the aid of Table 2 however, we can, in each individual case, ascertain between which tubes the boundaries lay, and which series was used, as the same figure does not occur twice in the table.

If the activity of the enzyme solution is slight, then the tubes where the boundary lies will contain a relatively large amount of enzyme solution. In cases where the hydrogen ion concentration of this solution differs in any marked degree from that of

the buffer solution, the result will be, that the concentration of hydrogen ions in the various tubes does not amount to the value contemplated. This effect will be greatest in the first tube, which contains the greatest amount of enzyme, and will then decrease from each tube to the next. Using Series A, this is prevented by giving the buffer solution in tubes 1 and 2 a composition differing somewhat from that of the rest. As the enzyme in the following tubes is obtained by dilution of that in tube N° 2, there is no need for special treatment of these tubes. In series B, the quantities of enzyme employed are so small that there will be no dislocation of the hydrogen ion concentration.

b. The activity of the saccharogenamylase is determined as follows: 25 cc of 2 % solution of soluble starch is placed in a flask together with 10 cc buffer solution, unless otherwise stated, a  $\frac{1}{15}$  molar phosphate solution «0.12 secondary». The flask is placed in a water thermostat at 38°. When the solution has attained the temperature of the water, 5 cc of a suitably diluted enzyme solution is added. Unless otherwise stated, 2 cc of the enzyme to be investigated has been diluted to 50 cc volume. In this case also, the resulting concentration of hydrogen ions will be  $p_H = 5.4$ . After 30 minutes, 10 cc are removed and transferred by a pipette to 50 cc copper solution. The reduction capacity is determined by titration according to Bang's first method, the hydroxylamin method<sup>1)</sup> and is expressed in mg maltose. A control experiment is carried out in the same way, but with boiled enzyme solution. The difference between the quantity of maltose in the sample and the control sample is taken as a measure of the activity of the saccharogenamylase. The reduction capacity depends in the first place on the formation of maltose, but it seems to me likely that it in certain cases depends, at any rate to some extent, on the formation of reducing carbohydrate of higher molecular weight than maltose.

As Bang's method is only graduated for glucose, it was necessary to effect a graduation for maltose. A number of titrations were made with known quantities of maltose, and Table 3 compiled from the values found. In drawing up this table, I have, in accordance with the proposal put forward by Jessen-Hansen<sup>2)</sup> noted under cc not the number of cubic centimetres of hydroxyl-

<sup>1)</sup> Biochem. Zeitschr. **2**, 271 (1907); **11**, 538 (1908); **32**, 443 (1911).

<sup>2)</sup> Comptes-rendus du Lab. Carlsberg **7**, 199 (1909).

amin solution consumed, but the difference between the quantity of hydroxylamin solution consumed in standardization of the copper solution by the hydroxylamin solution according to Bang, and the quantity consumed in carrying out the analysis. Under the heading of maltose is given no. of mg waterfree maltose. Table 4 gives the observations on which Table 3 is based. The difference between the weighed quantities and those calculated according to Table 3 only amounts in two cases to 0.4 mg. Reckoning from the whole series, the mean error of the individual observations is calculated as 0.19 mg.

Table 3.

cc	mg maltose	cc	mg maltose	cc	mg maltose	cc	mg maltose
1.0	1.0	13.0	20.2	25.0	39.8	36.0	59.5
2.0	2.6	14.0	21.9	26.0	41.4	37.0	61.4
3.0	4.2	15.0	23.5	27.0	43.0	38.0	63.4
4.0	5.8	16.0	25.1	28.0	44.7	39.0	65.5
5.0	7.4	17.0	26.7	29.0	46.5	40.0	67.5
6.0	9.0	18.0	28.3	30.0	48.2	41.0	69.6
7.0	10.6	19.0	30.0	31.0	50.0	42.0	71.7
8.0	12.2	20.0	31.6	32.0	51.9	43.0	73.8
9.0	13.8	21.0	33.2	33.0	53.7	44.0	76.0
10.0	15.4	22.0	34.8	34.0	55.6	45.0	78.2
11.0	17.0	23.0	36.5	35.0	57.5	46.0	80.4
12.0	18.6	24.0	38.1				

Table 4.

cc	maltose found	maltose weighed	diffe- rence	cc	maltose found	maltose weighed	diffe- rence
1.1	1.2	1.2		14.9	23.3	22.9	- 0.4
1.9	2.5	2.4	- 0.1	16.6	26.1	26.2	+ 0.1
2.6	3.6	3.6		16.8	26.4	26.4	
3.3	4.7	4.7		20.1	31.7	31.7	
3.2	4.5	4.8	+ 0.3	20.2	31.9	32.2	+ 0.3
3.9	5.6	5.3	- 0.3	22.2	35.2	34.9	0.3
4.0	5.8	6.0	+ 0.2	23.2	36.8	36.9	+ 0.1
4.8	7.1	7.1		23.8	37.8	37.5	- 0.3
5.6	8.4	8.3	- 0.1	27.3	43.5	43.7	+ 0.2
6.0	9.0	9.0		31.0	50.0	50.0	
6.1	9.2	9.2		32.2	52.2	52.4	+ 0.2
6.4	9.6	9.5	- 0.1	32.4	52.6	52.6	
7.4	11.2	11.4	+ 0.2	32.5	52.8	52.8	
8.8	13.5	13.9	+ 0.4	34.3	56.2	56.3	+ 0.1
10.2	15.7	15.8	+ 0.1	37.0	61.4	61.1	- 0.3
11.4	17.7	17.5	- 0.2	37.4	62.2	62.5	+ 0.3
11.8	18.3	18.3		41.1	69.8	69.8	
13.4	20.9	21.1	+ 0.2	45.2	78.6	78.6	



c. The concentration of hydrogen ions was determined electrometrically according to Sørensen, by passing hydrogen through the solution, but in some cases, the quinhydrone electrode method was used, and in such cases, this is stated. Experiments have shown that the quinhydrone electrode, within the range where it can be employed, gives the same value as the hydrogen electrode.

### 3. Initial material.

The initial material employed was prepared in the following manner. Green malt is passed through a mincing machine and steeped with an equal weight of water in an ice box for abt. 20 hours. It is then pressed in a Büchner press, and the liquid thus extracted is filtered. After addition of toluol, the filtrate thus obtained can be kept in an ice box for months without losing too much of its activity<sup>1)</sup>.

### 4. Alteration of Malt Diastase by Dialysis.

To 200 cc malt solution,  $p_H = 4.20$ , was added toluol, and the whole dialysed through collodium in an apparatus according to Sørensen<sup>2)</sup> with distilled water as the outer liquid. The whole apparatus was immersed in ice water. In the outer vessel, a constant under-pressure was maintained, counteracting the osmotic pressure of the inner liquid, so that the volume of the inner liquid was not increased, but on the contrary, decreased. After 15 days, the dialysis was stopped. The volume of the inner liquid then amounted to 62 cc. After dilution with water to a volume of 200 cc, the activity of the inner liquid was investigated, and compared with that of non-dialysed malt solution. The result will be seen from the following:

	mg maltose	X		mg maltose	X
Non dialysed ...	44.4	495	Dialysed....	23.0	6

In the course of dialysis, then, there is a reduction both in the activity of saccharogenamylase and in that of dextrinogenamylase, but in an essentially different degree. The saccharogenamylase has been reduced to something like half, whereas of the dextrinogenamylase, there remains only 1 or 2 %.<sup>3)</sup>

<sup>1)</sup> Cf. p. 10.

<sup>2)</sup> Studies on Proteins. Comptes rendus du Lab. Carlsberg 12, 26 (1917).

<sup>3)</sup> The activity of the dextrinogenamylase is not restored by addition of sodium chloride.

### 5. Stability of the Enzyme at different concentrations of hydrogen ions, and different temperatures.

One series of experiments comprises those carried out at different concentrations of hydrogen ions, but at the same temperature. These experiments were made partly in an ice box, partly in a water thermostat. In the former case, the temperature could not be maintained perfectly constant, but varied between abt.  $5^{\circ}$  and  $8^{\circ}$ . In the water thermostat, experiments were made at  $38^{\circ}$ ,  $50^{\circ}$ ,  $60^{\circ}$ ,  $70^{\circ}$ ,  $75^{\circ}$  and  $80^{\circ}$ .

The method employed was as follows. 25 cc of malt solution was first diluted with water, and to this was then added 0.1 N hydrochloric acid or caustic soda, so as to bring the volume up to 100 cc and give the required concentration of hydrogen ions. At the five higher temperatures, both the water and the hydrochloric acid or caustic soda were previously warmed. After a suitable lapse of time, samples were taken, and examined as described in Chap. 2. These investigations of the activity of the sample were thus always carried out at  $38^{\circ}$ , and  $p_H = 5.4$ , irrespective of the previous temperature and concentration of hydrogen ions in the enzyme.

The values given for the activity of both enzymes at the time  $t = 0$  have not been separately determined in each case, but are uniform for all the experiments in a series. They are obtained by diluting the original malt solution to 4 times its volume, and then proceeding as usual. This method is open to some objections, as not all the experiments of a series were started on the same day. The error thus arising however, is so slight as to be of no importance in this connection, as an enzyme solution, prepared in the manner above described and preserved with toluol in an ice box, loses its activity very slowly indeed. By way of example we may take the malt solution used for all experiments at  $38^{\circ}$  and over. The solution was prepared on the  $9/8$  1923. Its activity at different dates will be seen from the following table:

	maltose	X	Y			maltose	X	Y
28. 8. 23..	72.5	296	1770	15. 2. 24..		230	1360	
7. 12. 23..	69.5			16. 2. 24..	65.2			
11. 12. 23..		296	1770	14. 3. 24..	61.8			

In the following, the time is stated in days of 24 hours, and with never more than 3 significant figures. The times for deter-

mination of hydrogen ion concentration, however, are exact only to the nearest 24 hours.

Three complete series with different initial material were made at ice box temperature. These series, which must be regarded as of fundamental importance in the present investigations, agree well one with another. There was a slight difference in the manner in which they were carried out, the hydrogen ion concentration in series II and III, which were made first, being determined only once in the course of each experiment. This however, proved insufficient, as the concentration of hydrogen ions altered perceptibly during the course of the experiment. In series I, which was in reality carried out later than II and III, the concentration of hydrogen ions was therefore determined several times in the different experiments. This series is here given first.

The series are indicated in Roman numerals, the individual experiments in ordinary figures. The tables show the time, and the quantities mg maltose, X and Y, the signification of which will be apparent from Chapt. 2. The  $p_H$  is also stated: and at the head of each table is given the quantity of hydrochloric acid or caustic soda employed, with the date when the experiment was commenced.

I: 1.					I: 2.				
25 cc 0.1 n HCl					20 cc 0.1 n HCl				
13. 12. 1920					13. 12. 1920				
days	maltose	X	Y	$p_H$	days	maltose	X	Y	$p_H$
0	51.3	384	2250		0	51.3	384	2250	
0.003	1.7	0	0	2.53	0.003	28.8	6	190	
					0.063	1.9	0	12	2.83

I: 3.					I: 4.				
15 cc 0.1 n HCl					12.5 cc 0.1 n HCl				
14. 12. 1920					19. 12. 1920				
days	maltose	X	Y	$p_H$	days	maltose	X	Y	$p_H$
0	51.3	384	2250		0	51.3	384	2250	
0.003	40.1				0.020		24	380	
0.008		6	380		0.033	39.8			
0.042	39.3	6	380		0.952		6	380	
0.135	37.3	3	190	3.28	0.992	37.7			3.57
0.958	30.1				2.93	35.5			
1.06		0	190		7.83		0	100	
2.19	19.5				7.95	34.5			
3.17	14.1	0	100		16.0	32.9			
4.07	10.3				22.0	31.3			
6.11	5.1				28.8	26.2			
15.1	3.2				32				3.59
36.9	2.1			3.35	37.9	25.6			
					60.8	19.4			
					139				3.71
					146	0.0			

I: 5.					I: 7.				
10 cc 0.1 n HCl					5 cc 0.1 n HCl				
16. 12. 1920					16. 12. 1920				
days	maltose	X	Y	P <sub>H</sub>	days	maltose	X	Y	P <sub>H</sub>
0	51.3	384	2250		0	51.3	384	2250	
0.003		190	1500		0.015	50.2			
0.016	48.1				1.02		230	1360	4.68
0.227		24	770		1.20	48.7			
1.14		12	380	3.88	1.99		247	1460	
1.20	40.4				2.18	49.2			
1.93		6	190		6.08	47.5			
4.14	40.1				11.9		247	1460	
4.94		6	190		13.1	47.1			
11.0		6	190		23.0	46.5	192	1130	
11.1	39.1				34.0		192	885	4.70
21.9		3	190		39.2	45.0			
33.9		0	100	3.89	57.9		115	679	
39.2	34.1				63.9	37.6			
63.9	27.2				126		48	380	
142				3.94	142				4.61
149	0.0				149	24.5			

I: 6.					I: 8.				
7.5 cc 0.1 n HCl					2.5 cc 0.1 n HCl				
16. 12. 1920					18. 12. 1920				
days	maltose	X	Y	P <sub>H</sub>	days	maltose	X	Y	P <sub>H</sub>
0	51.3	384	2250		0	51.3	384	2250	
0.015	49.2				0.162		296	1360	
1.11		192	1460	4.26	0.347	49.6			5.61
1.20	47.0				21.1	50.6			
1.95		148	885		25.1		230	1040	
2.10	45.7				39.2	46.6			
4.14	44.0				47				5.39
5.95		74			56.1		247	1130	
11.1	40.2	48	770		62.1	42.8			
23.1		12	380		125		192	885	
33.9		6	190	4.26	140				4.98
39.2	37.0				147	36.2			
56.1		6	190						
63.9	30.8								
126		0	24						
142				4.20					
149	0.0								

I: 9.					I: 12.						
18. 12. 1920					7.5 cc 0.1 n NaOH 19. 12. 1920						
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>		
0	51.3	384	2250	6.35	0	51.3	384	2250	7.78		
0.347	49.2				0.843		296	2250			
1.06		384	2250		2.92	48.8					
21.1	49.0				7.96	47.8					
33.1		230	1360		8.79		296	1770			
38.3	46.4			6.21	11				7.45		
55					22.0	43.9					
58.0		247	1460		37.0	42.2	230	1360			
66.1	42.6				57.9		192	1130			
125		192	885		59						
140				5.81	64.7	38.3			7.12		
147	36.4				136		192	885			
					139						
					146	24.8					
I: 10.					I: 13.						
2.5 cc 0.1 NaOH 18. 12. 1920					10 cc 0.1 n NaOH 21. 12. 1920						
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>		
0	51.3	384	2250	6.77	0	51.3	384	2250	8.16		
0.347	51.3				1.03		296	1770			
1.12		296	2250		1.17	47.9					
21.1	48.3				6.15	46.5					
37.2		230	1770		7.02		296	1360			
38.3	47.4			6.51	8				7.86		
55					13.2	39.7					
58.1		247	1130		20.2	39.5					
66.1	42.6				35.1		230	1360			
127		192	885		35.2	34.6					
140				6.39	56.1		192	1130	7.45		
147	34.1				64.1	31.2					
					138						
					142		148	679			
					144	17.8					
I: 11.											
5 cc 0.1 n NaOH 19. 12. 1920											
days	maltose	X	Y	p <sub>H</sub>							
0	51.3	384	2250	7.25							
0.809		296	2250								
0.990	48.7										
7.95	47.9										
19.8	47.9										
35.9		230	1770	6.90							
37.0	43.0										
56.8		192	1130								
59											
64.7	40.5										
136		192	885	6.65							
139											
146	26.1										

I: 14.					I: 16.				
12.5 cc 0.1 n NaOH					20 cc 0.1 n NaOH				
29. 12. 1920					29. 12. 1920				
days	maltose	X	Y	pH	days	maltose	X	Y	pH
0	51.3	384	2250	8.48	0	51.3	384	2250	9.33
0.354	49.2				0.354	45.1			
1.31	45.9				1.31	44.8			
5.31	41.2				5.01		230	1360	9.12
12.2		296	1360		7.38	35.6			
12.3	41.7				19.2	29.0			
27.1	37.4				19.4		192	1130	8.69
27.3		230	1360		27.1	22.2			
44.2		192	1130		44.1		148	885	
49				7.84	49				8.37
55.1	26.3				56.7	14.0			
130				7.54	130				
134		148	679		135		96	340	
136	11.4				136	2.2			

I: 15.					I: 17.				
15 cc 0.1 n NaOH					25 cc 0.1 n NaOH				
29. 12. 1920					4. 1. 1921				
days	maltose	X	Y	pH	days	maltose	X	Y	pH
0	51.3	384	2250	8.89	0	51.3	384	2250	9.69
0.354	45.8				0.250	43.0			
1.31	44.1				1				
5.17		230	1360		2.92		230	1360	9.16
5.31	40.1				3.21	34.2			
7.38	37.7				6.20	25.5			
19.2	31.0				13.0	20.7			8.78
27.1	26.6				13.2		192	1130	
29.1		192	1460		21.0	15.4			
44.2		192	1130		37.1		148	885	8.78
49				8.20	44				
56.3	18.5				50.2	8.0			
130				7.90	124				
135		124	563		130	0.7	57	261	
136	6.6								

I : 18.					I : 20.				
30 cc 0.1 n NaOH					40 cc 0.1 n NaOH				
4. I. 1921					19. I. 1921				
days	maltose	X	Y	pH	days	maltose	X	Y	pH
0	51.3	384	2250	9.98	0	51.3	384	2250	10.53
0.250	38.5				0.118	11.2			
1.25	26.4	296	1360		0.215		148	885	
2.90		230	1360		0.899		124	729	
3.21	13.3				1.03	3.5			
6.13		192	1130	9.45	2				9.91
6.20	7.0				3.00	2.9			
13.0	6.7				3.20		96	729	
13.2		148	885		7.15	2.0	96	729	
21.0	3.8				22.0		48	380	
37.1		96	442	9.09	29				9.44
44					109				
50.2	3.5				114		6	100	
124									
130		48	380						

I : 19.					I : 21.				
35 cc 0.1 n NaOH					45 cc 0.1 n NaOH				
19. I. 1921					21. I. 1921				
days	maltose	X	Y	pH	days	maltose	X	Y	pH
0	51.3	384	2250	10.25	0	51.3	384	2250	10.91
0.118	30.6				0.059	9.1			
0.160		247	1460		0.066		124	729	
1.00		192	885		1.11	0.7			
1.03	9.6				1.32		48	190	
2				9.70	3.11		12	100	9.21
3.00	6.1				5.09		12	100	
3.17		192	885		20.1		0	24	
7.15	5.7								
8.15		148	885						
22.0		124	729	9.21					
29									
35.2	1.1								
109									
115		48	380						

I: 22.					I: 23.				
50 cc 0.1 n NaOH					55 cc 0.1 n NaOH				
25. I. 1921					26. I. 1921				
days	maltose	X	Y	p <sub>H</sub> <sup>1)</sup>	days	maltose	X	Y	p <sub>H</sub> <sup>1)</sup>
0	51.3	384	2250	11.17	0	51.3	384	2250	11.37
0.097	1.6				0.003	24.8			
0.104		24	190		0.007		100	770	
1.00		0	24		0.038	1.2			
					0.045		24	190	
					1.08		0	0	

<sup>1)</sup> p<sub>H</sub> determined in another solution of the same composition.

At a concentration of hydrogen ions of  $p_H = 2.5$ , both enzymes are destroyed almost entirely in five minutes, but at  $p_H = 2.8$  we already find the saccharogenamylase noticeably more resistant. Nevertheless, it is half destroyed in 5 minutes, and almost completely in 1½ hours. At  $p_H = 3.3$ , the period required to halve the saccharogenamylase has already risen to 24 hours, and at  $p_H = 3.6$  it takes about a month, the dextrinogenamylase being throughout very unstable. Not until a  $p_H = 3.9$  is the stability of the dextrinogenamylase increased to any considerable extent. At this concentration of hydrogen ions, the destruction of saccharogenamylase takes place very slowly indeed. We find, for instance, for the times 1.20, 4.14 and 11.1, the values 40.4, 40.1 and 39.1 mg maltose. In Exp. I: 8, with an initial concentration of hydrogen ions of  $p_H = 5.6$ , both enzymes have attained their maximal stability. Expp. I: 9 and I: 10, starting with  $p_H = 6.35$  and 6.8, respectively, differ but inconsiderably from I: 8, but in I: 11, which starts at  $p_H = 7.25$ , we already find a decrease in the stability. This decrease is quite distinct in the case of the saccharogenamylase, but lies within the limits of experimental error for the dextrinogenamylase. In the experiments next following: I: 12 to I: 15, inclusive, with initial  $p_H = 7.8$  to 8.9, the saccharogenamylase is destroyed more and more rapidly, while the stability of the dextrinogenamylase is reduced only to an inconsiderable extent. At still higher  $p_H$  values, the stability of both enzymes is still further reduced, the dextrinogenamylase however, being now throughout more stable than the saccharogenamylase. In the last of the experiments, with  $p_H = 11$  and over, both enzymes are very soon destroyed.



It will thus be seen, from these experiments, that we can obtain a saccharogenamylase solution more or less free from dextrinogenamylase by exposing the enzyme to acid reaction  $p_H$  less than 4, for a suitable period, in an ice box. Conversely, we can obtain a solution of dextrinogenamylase more or less free from saccharogenamylase by exposing the enzyme to alkaline reaction. In the latter case, however, better results are obtained by working at a higher temperature. The method described in the following (p. 42) for the preparation of saccharogenamylase solution is based exclusively on experiments of the nature above indicated.

II: 1.					II: 3.					
20 cc 0.1 n HCl					12.5 cc 0.1 n HCl					
14. 7. 1920					5. 7. 1920					
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>	
0	66.0	495	2250	2.98	0	66.0	495	2250	3.77	
0.072		0	12		0.105		24	190		
0.147	1.4				0.170	47.1				
					1.11	45.2				
					1.97		24	190		
				4.00		12	190			
				4.08	44.3					
				5						
				6.97			12	100		
				7.18	44.1					
				23.2	39.4					
				25.1		6	100			

II: 2.					II: 4.				
15 cc 0.1 n HCl					10 cc 0.1 n HCl				
6. 7. 1920					1. 7. 1920				
0	66.0	495	2250	3.39	0	66.0	495	2250	4.26
0.059		24	380		0.113		296	1770	
0.097	47.1				0.966		115	521	
0.934		12	190		1.10	47.4			
1.07	39.8				1.98		57	261	
2.95		6	48		2.08	45.1			
3.07	33.4				3.99		24	190	
4					8.08	45.3			
5.93		0	48		11.1		24	190	
6.17	23.6				13.2	44.6			
10.2	16.7				28.2	44.0			
22.2	10.1				29.1		24	190	

II: 5.					II: 9.					
7.5 cc 0.1 n HCl					2.5 cc 0.1 n NaOH					
1. 7. 1920					28. 6. 1920					
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>	
0	66.0	495	2250	4.80	0	66.0	495	2250	6.84	
0.990		230	1040		6					
1.10	58.5				17.8		495	1770		
2.09	57.2				18.1	60.2				
2.99		247	1130		35.0	57.3				
4.17	54.5				36.8		384	1360		
9.07		247	1130							
13.2	54.3									
29.2		203								
32.1	48.8									
II: 6.					II: 10.					
5 cc 0.1 n HCl					5 cc 0.1 n NaOH					
28. 6. 1920					28. 6. 1920					
0	66.0	495	2250	5.36	0	66.0	495	2250	7.25	
2.87		296	1360		6					
3.98	58.5				17.9		495	1770		
5					18.1	54.1				
6.96		317			37.0	50.8	296	1360		
15.8		317								
16.0	58.4									
34.9		317	1460							
35.0	59.4									
II: 7.					II: 11.					
2.5 cc 0.1 n HCl					7.5 cc 0.1 n NaOH					
28. 6. 1920					28. 6. 1920					
0	66.0	495	2250	5.71	0	66.0	495	2250	7.64	
5					1.07	64.6				
16.8		384	1770		4.97	59.4				
18.1	64.1				6					
35.0	64.5	384	1770		16.0	42.8				
					18.0		384	1770		
					30.1	37.5				
					36.1		296	1360		
II: 8.					II: 12.					
28. 6. 1920					10 cc 0.1 n NaOH					
0	66.0	495	2250	6.47	0	66.0	495	2250	7.79	
6					1.07	62.2				
16.9		495	1770		4.97	52.0				
18.1	63.2				10					
35.0	63.1				11.0	43.4				
36.8		384	1770		18.0		296	1360		
					21.0	29.4				
					30.1	25.2				
					36.1		230	1360		

II: 13.					II: 16.					
12.5 cc 0.1 n NaOH					25 cc 0.1 n NaOH					
28. 6. 1920					25. 6. 1920					
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>	
0	66.0	495	2250	8.02	0	66.0	495	2250	9.47	
2.06	50.1				0.235	47.5				
3.98	37.8				0.918	23.0				
8.00	27.5				3.18	7.7				
10					3.95		247	1460		
14.1	15.6			5.16	7.2					
18.8		317	1460	12.1		192	1130			
30.1	11.5			15						
36.0		247	1130	24.1	7.2	192	885			
				34.1	5.8					
				39.0		148	885			

II: 14.					II: 17.				
15 cc 0.1 n NaOH					30 cc 0.1 n NaOH				
28. 6. 1920					25. 6. 1920				
0	66.0	495	2250	8.44	0	66.0	495	2250	9.83
1.07	49.2				0.236	21.0			
2.06	37.1				0.919	8.9			
3.98	24.9				4.05		247	1460	
8.00	14.9				5.17	6.0			
10					6.13		192	1130	
14.1	10.7				11.1		124	729	
18.9		317	1460		12.1	4.2			
31.0	8.3				15				
36.0		247	1130		19.1		124	729	
				24.1	4.9				
				34.1	5.0				
				38.2		124	729		

II: 15.					II: 18.				
20 cc 0.1 n NaOH					35 cc 0.1 n NaOH				
28. 6. 1920					25. 6. 1920				
0	66.0	495	2250	8.97	0	66.0	495	2250	10.18
1.07	37.0				0.106	10.9			
2.07	23.9				0.245		190		
3.98	13.9				0.921	6.5			
8.00	10.0				3.18	1.9			
8.12		247	1130		4.09		124	729	
10					7.22		96	563	
14.1	9.1				10.2		48	190	
19.0		247	1130		15				
31.0	6.7				17.1		48	190	
36.0		192	885		24.1		24	100	
					36.0		24	100	

II: 19.					III: 3.				
40 cc 0.1 n NaOH					12.5 cc 0.1 n HCl				
25. 6. 1920					18. 7. 1919				
days	maltose	X	Y	P <sub>H</sub>	days	maltose	X	Y	P <sub>H</sub>
0	66.0	495	2250	10.39	0	47.0	247	1130	3.73
0.099		100	770		0.178	31.2			
0.107	4.0				0.190		12	190	
0.303		100	770		1.07	30.3			
0.922	1.9				1.19		12	190	
1.07		24	190		3.10	29.0			
2.97		12	100		4.94		6	100	
4.94		6	48		7.11	26.4			
7.19		3	24		11.1	21.9	3	100	
10.1		0	12		17.1	16.8			
15					18.1		0	24	

II: 20.					III: 4.					
45 cc 0.1 n NaOH					10 cc 0.1 n HCl					
25. 6. 1920					15. 7. 1919					
0	66.0	495	2250	10.75	0	47.0	247	1130	4.03	
0.047		48	380		1.13		48	380		
0.107	0.8				2.01		12	380		
0.269		0	0		2.11	36.7				
15					5.00		12	190		
					13.2		12	190		
III: 1.					14.1	33.7				4.40
17.5 cc 0.1 n HCl					22.2	32.9				
18. 7. 1919					24.1		6	100		
0	47.0	247	1130	42.1		6	100			
0.136	11.6	3	48	43.1	30.3					
1.07	2.2			342		3	48			
1.16		0	12	386	18.3					
3.10	0.0									
4.94		0	0							

III: 2.					III: 5.				
15 cc 0.1 n HCl					7.5 cc 0.1 n HCl				
18. 7. 1919					15. 7. 1919				
0	47.0	247	1130	3.46	0	47.0	247	1130	4.40
0.158	27.4				2.11	42.1			
0.165		6	100		2.19		190	1500	
1.07	23.0				9.14		96	729	
1.16		3	48		21.2		96	442	
3.10	13.3				22.1	37.8			
4.97		0	24		42.1		57	340	
7.11	10.0				43.1	37.2			
11.1	7.1				329		6	48	
17.1	6.6				337	21.1			
18.1		0	24						

## III: 6.

5 cc 0.1 n HCl		15. 7. 1919		
days	maltose	X	Y	P <sub>H</sub>
0	47.0	247	1130	4.95
2.11	44.0			
13.1	42.6			
15.0		115	679	
22.1	39.6			
42.1		124	563	
43.1	39.8			
328		12	190	
335	19.6			

## III: 10.

7 cc 0.1 n NaOH		15. 7. 1919		
days	maltose	X	Y	P <sub>H</sub>
0	47.0	347	1130	7.90
2.20	43.8			
7.17	42.0			
15.1	37.0			
16.2		192	885	
43.1	32.3			
45.1		148	885	
337	7.6	48	380	

## III: 7.

2.5 cc 0.1 n HCl		15. 7. 1919		
0	47.0	247	1130	5.88
2.20	45.9			
15.1	40.5	148	885	
42.2		148	679	
43.1	41.4			
334		48	380	
335	22.9			

## III: 11.

10 cc 0.1 n NaOH		21. 7. 1919		
0	47.0	247	1130	8.19
0.164	44.2			
1.22	44.1			
9.19	34.9			
11.2		192	885	
16.2	34.2			
38.2	26.7			
39.2		115	679	
330		48	380	
331	6.6			

## III: 8.

		15. 7. 1919		
0	47.0	247	1130	6.58
2.20	47.0			
15.1	40.6			
16.0		148	679	
42.2		148	679	
43.1	40.4			
335	17.4	100	770	

## III: 12.

15 cc 0.1 n NaOH		21. 7. 1919		
0	47.0	247	1130	8.79
1.22	44.8			
9.19	24.1			
12.0		115	679	
38.2	4.0			
40.1		96	563	
330		12	100	

## III: 9.

3.5 cc 0.1 n NaOH		15. 7. 1919		
0	47.0	247	1130	7.27
2.20	46.3			
15.1	38.6			
16.1		148	679	
43.1	35.5			
45.1		115	679	
335	14.1	48	380	

## III: 13.

20 cc 0.1 n NaOH		21. 7. 1919		
0	47.0	247	1130	9.35
0.168	44.2			
1.22	41.7			
8.15	28.7			
12.1		148	885	
16.2	19.6			
38.2	3.8			
40.0		74	563	
330		12	100	

III: 14.					III: 17.				
25 cc 0.1 n NaOH					40 cc 0.1 n NaOH				
24. 7. 1919					24. 7. 1919				
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>
0	47.0	247	1130	9.86	0	47.0	247	1130	10.84
0.118	41.8				0.122	9.9			
1.12	32.1				0.194		100	1500	
4.07	22.5				0.929		100	770	
11.1	13.6	148	885		1.11	3.2			
35.1	2.4				4.07	1.7	48	190	
37.0		74	563		11.1	1.5	48	190	
328		12	100		34.0	0.0	24	190	

III: 15.					III: 18.				
30 cc 0.1 n NaOH					45 cc 0.1 n NaOH				
24. 7. 1919					29. 7. 1919				
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>
0	47.0	247	1130	10.21	0	47.0	247	1130	11.03
0.119	35.2				0.125	2.6			
1.12	14.5				0.140		48	380	
4.07	8.3				1.14	0.0			
11.1	4.5	124	729		1.18		0	100	
35.1	2.1				2.16		0	48	
37.0		57	261		6.97		0	24	
					31.0		0	12	

III: 16.					III: 19.				
35 cc 0.1 n NaOH					50 cc 0.1 n NaOH				
24. 7. 1919					30. 7. 1919				
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>
0	47.0	247	1130	10.55	0	47.0	247	1130	11.26
0.121	25.1				0.128		0	48	
1.12	4.3				0.134	0.0			
4.08	3.3				1.15		0	0	
11.1	2.7								
12.0		96	442						
35.1	1.0								
37.1		24	100						

The experimental series II and III agree well with series I. There seems, however, to be a slight difference between the experiments with alkaline reaction, the stability of the saccharogen-*amylase* being relatively highest in Series I and relatively lowest in Series II. This, however, is only a difference of degree, and is due, at any rate in part, to the fact that the determination of hydrogen ions is not altogether comparable in the different series. In series II and III, only a single determination of the concentration of hydrogen ions was made for each experiment, and this was done, for instance, in the alkaline solutions of series II, after

the lapse of 10 or 15 days. But by this time, a considerable alteration will have taken place in the concentration of hydrogen ions, tending towards lower  $p_H$ . If we were to disregard this fact, we should find ourselves comparing experiments in the different series which are properly not comparable. We must, therefore, compare for instance Exp. I : 18 with II : 16 and III : 14, not with II : 17; Exp. I : 19 should be compared with II : 17 and III : 15, not with II : 18 etc.

Even making due allowance for this, however, there is still a slight difference, showing that other factors besides temperature and concentration of hydrogen ions can also affect the stability of the enzymes. The dialysis experiments in Chap. 4 also show that the quantity of dialysable matter contained in the solutions is of importance to the stability of the dextrinogenamylase especially, but also to that of the saccharogenamylase. It should be noted that the temperature in these experiments, which were carried out in an ice box, was not completely constant. A slight alteration in the temperature may possibly be of more importance in its effects upon stability in the alkaline solutions than in the neutral and acid solutions.

The following series, IV to IX inclusive, were carried out at higher temperature, which is stated at the head of each table. As regards the experimental method here employed, there is a slight difference compared with the preceding series. The samples were, it is true, taken at the times stated, but were then, after cooling with ice water and in some cases, after addition of a small quantity of phosphate buffer, kept for a short time in the ice box — a matter of some few hours at the outside — until it was possible to test the activity of the enzyme.

IV : 1.					IV : 2.				
15 cc 0.1 n HCl      38°      27. 2. 24					12.5 cc 0.1 n HCl      38°      22. 2. 24				
days	maltose	X	Y	$p_H$	days	maltose	X	Y	$p_H$
0	65.2	230	1360	3.60	0	65.2	230	1360	3.90
0.042	3.9	0	24		0.042	38.5	0	190	
0.125	0.0				0.125	24.9	0	100	
					0.250	14.9			
					1.00	1.8			

IV: 3.					IV: 4.					IV: 5.					IV: 6.				
10 cc 0.1 n HCl 38° 20. 2. 24					7.5 cc 0.1 n HCl 38° 18. 2. 24					5 cc 0.1 n HCl 38° 14. 2. 24					2.5 cc 0.1 n HCl 38° 14. 2. 24				
days	maltose	N	V	pH	days	maltose	N	V	pH	days	maltose	N	V	pH	days	maltose	N	V	pH
0	65.2	230	1360		0	65.2	230	1360		0	65.2	230	1360		0	65.2	230	1360	
0.125	46.1	48	380		0.125	61.4				0.125	62.6				0.250	63.4			
0.250	40.6	12	190		0.250	57.0	190	1500		0.250	60.5				1.00	61.7			
1.00	28.2	0	48	4.16	1.00	50.4	100	770	4.49	1.00	58.4	190	1500		2.00	61.3			5.61
2.00	19.6				2.00	45.0	48	380		2.00	56.0	190	1500	4.89	4.00	60.8			
3.00	14.1				3.00	42.0	24	380		4.00	51.7	190	1500		6.00		190	1500	
5.00	6.5				4.00	40.0	6	190		5.00		190	1500		7.00	55.6			
7.00	3.5				5.00	36.4	3	190	4.56	6.00		100	770		9.00		190	1500	
9.00	2.7			4.14	7.00	32.4	0	100		7.00	47.1				11.0	49.7	190	1500	5.63
15.0	0.2				10.0	23.9				11.0	39.6	48	380	4.92	12.0		190	1500	
20.0				4.15	12.0	23.2				14.0		48	380		13.0		190	1500	
					16.0	18.4				15.0	33.5	48	380		14.0		190	1500	
					24.0	11.8				16.0		24	380		15.0	43.0	190	1500	
					29.0	10.7				19.0		24	190		16.0		190	1500	
					35.0	7.7			4.76	20.0	27.4	12	190		18.0	33.7	100	1500	
					49.0	3.8				25.0		12	100		20.0				
										27.0		6	100		28.0	24.6			
										28.0	18.6				30.0		100	770	
										33.0	15.6	3	100		32.0		48	380	
										39.0	10.7	0	48		33.0	20.2			
										53.0	6.2			4.86	39.0	13.6	48	380	
															53.0	8.6			
															54.0		24	190	5.65



IV: 7. 38°					IV: 8. 25 cc 0.1 n NaOH 38°					IV: 9. 5 cc 0.1 n NaOH 38°					IV: 10. 7.5 cc 0.1 n NaOH 38°				
days	maltose	N	Y	pH	days	maltose	N	Y	pH	days	maltose	N	Y	pH	days	maltose	N	Y	pH
0	65.2	230	1360		0	65.2	230	1360		0	65.2	230	1360		0	65.2	230	1360	
0.250	63.4				0.125	64.6				0.125	64.0				0.125	59.3			
1.00	60.7				0.250	64.2				0.25	60.3				0.250	54.8			
2.00	60.5			6.36	1.00	59.7			6.92	1.00	53.3				1.00	35.5			
4.00	57.9				2.00	58.1				2.00	44.6				2.00	25.3			7.82
6.00		190	1500		4.00	46.9				3.00	38.8				3.00	19.1			
7.00	52.2				6.00	43.4				4.00	33.1				4.00	14.9	190	1500	
8.00		190	1500		8.00	36.4				5.00	27.7	190	1500		6.00	10.5	190	1500	
11.0	43.2	190	1500		10.0	32.4	190	1500	6.81	7.00	21.8	190	1500	7.04	7.00		190	1500	7.49
13.0		190	1500	6.31	11.0		190	1500		8.00		190	1500		8.00	7.6	190	1500	
14.0		190	1500		12.0		190	1500		9.00		190	1500		9.00		100	770	
15.0	34.8	190	1500		13.0	24.2	190	1500		10.0	15.7	190	1500		11.0	6.3			
16.0		190	1500		14.0		190	1500		11.0		190	1500		16.0	4.6			
18.0		190	1500		15.0	21.9	100	1500		12.0	12.1	100	1500		20.0	3.8	48	380	
19.0		190	1500		19.0	17.3				16.0	9.4	100	770		24.0		48	380	7.08
20.0	25.6	100	770		27.0	11.4				21.0	7.7	100	770		27.0		48	380	
28.0	17.2				28.0		100	770		22.0		100	770		28.0	2.6			
33.0	14.0	100	770		31.0		100	770		25.0	5.9	100	770	6.83	34.0	1.3			
39.0	9.3	48	380		32.0	8.5				28.0		100	770		35.0		48	380	
53.0	5.3				38.0	5.9	48	380	6.44	29.0					49.0		24	190	6.65
54.0		48	380	5.93	52.0	5.1				35.0	3.2	48	380						
					53.0		48	380	6.39	49.0	2.6								
										50.0		48	380	6.57					

## IV: 11.

10 cc 0.1 n NaOH 38° 21. 2. 24

days	maltose	X	Y	P <sub>H</sub>
0	65.2	230	1360	
0.042	53.5			
0.125	41.0			
0.250	31.6			
1.00	12.8			
2.00	7.1			8.17
4.00	5.9	190	1500	
5.00		100	1500	7.94
8.00		100	770	
9.00	4.3	100	770	
11.0		100	770	
12.0		100	770	
13.0		48	770	
14.0	1.3			
18.0		48	380	
19.0		48	380	7.34
21.0		48	380	
23.0		48	380	
26.0		48	380	
33.0		24	190	7.15
47.0		24	190	6.95

## IV: 13.

15 cc 0.1 n NaOH 38° 28. 2. 24

days	maltose	X	Y	P <sub>H</sub>
0	65.2	230	1360	
0.042	21.8			
0.125	7.8			
0.250	6.5			
1.00	4.6	190	1500	8.67
2.00	4.6	190	1500	
4.00		100	770	
5.00		100	770	
6.00		100	770	
7.00	1.8	100	770	
11.0	1.5	48	770	
12.0		48	770	7.89
13.0		48	770	
19.0		48	380	
26.0		48	380	7.40
39.0		24	190	7.11

## IV: 12.

12.5 cc 0.1 n NaOH 38° 26. 2. 24

days	maltose	X	Y	P <sub>H</sub>
0	65.2	230	1360	
0.042	33.3			
0.167	13.9			
0.250	8.9			
1.00	4.6			8.39
2.00	4.6	190	1500	
3.00		100	770	
4.00	4.4	100	770	
6.00		100	770	
7.00		100	770	
8.00		100	770	
9.00	2.4	100	770	
13.0	2.1	48	770	7.62
18.0		48	380	
21.0		48	380	
28.0		48	380	7.29
42.0		24	190	7.03

## IV: 14.

20 cc 0.1 n NaOH 38° 3. 3. 24

days	maltose	X	Y	P <sub>H</sub>
0	65.2	230	1360	
0.042	8.3			
0.125	6.2			
0.250	5.8			
1.00	5.3	190	1500	9.09
2.00	4.0	190	1500	
3.00	3.2	100	770	
7.00	2.1	100	770	
8.00		100	770	8.38
9.00		48	380	
12.0		48	380	
15.0		48	380	
22.0		48	380	7.71
35.0		24	190	7.32

## IV : 15.

25 cc 0.1 n NaOH 38° 3. 3. 24

days	maltose	X	Y	P <sub>H</sub>
0	65.2	230	1360	
0.042	6.4			
0.125	5.9			
0.250	5.8			
1.00	4.5	190	1500	9.53
2.00	3.4	190	1500	
3.00	3.0	100	770	
7.00	1.9	100	770	
8.00		48	380	8.80
11.0		48	380	
12.0		48	380	
15.0		48	380	
22.0		24	380	8.05
35.0		24	190	7.61

## IV : 17.

35 cc 0.1 n NaOH 38° 10. 3. 24

days	maltose	X	Y	P <sub>H</sub>
0	65.2	230	1360	
0.042	4.3			
0.125	4.2			
0.25	3.2	190	1500	
1.00	1.8	100	770	10.01
2.00		48	380	
3.00		24	190	
4.00		24	190	
5.00		24	190	9.42
7.00		24	190	
8.00		24	190	
14.0		12	100	8.62
28.0		3	48	8.31

## IV : 16.

30 cc 0.1 n NaOH 38° 10. 3. 24

days	maltose	X	Y	P <sub>H</sub>
0	65.2	230	1360	
0.042	5.6			
0.125	5.0			
0.250	4.8	190	1500	
1.00	4.6	190	1500	9.81
2.00		100	770	
3.00		100	770	
4.00	2.9	100	770	
5.00		100	770	9.35
7.00		48	380	
8.00	1.2	48	380	
14.0		24	190	8.50
28.0		24	190	8.15

## IV : 18.

40 cc 0.1 n NaOH 38° 13. 3. 24

days	maltose	X	Y	P <sub>H</sub>
0	65.2	230	1360	
0.042	3.2	48	380	
0.083		24	190	
0.125	0.2	24	190	
0.250		6	100	10.37

## IV : 19.

45 cc 0.1 n NaOH 38° 14. 3. 24

days	maltose	X	Y	P <sub>H</sub>
0	65.2	230	1360	
0.042	0.0	0	0	10.72

V: 1.					V: 5.					
12.5 cc 0.1 n HCl		50°		6. 12. 23	6.25 cc 0.1 n HCl		50°		9. 1. 24	
days	maltose	N	Y	pH	days	maltose	N	Y	pH	
0	69.5	296	1770	3.90	0	69.5	296	1770	4.69	
0.007	6.4	3	48		0.021	58.0				
0.021	0.0	0	12		0.042	48.3				
0.042		0	3		0.083	36.5				
					0.167	21.5	190	1500		
					0.250	13.3	190	1500		
					0.333	9.9	100	770		
					0.500	5.7	48	380		
					1.00	1.6	12	100	4.69	
					1.50	0.5	6	48		
					2.00		0	12		
V: 2.					V: 6.					
10 cc 0.1 n HCl		50°		16. 1. 24	5 cc 0.1 n HCl		50°		11. 12. 23	
0	69.5	296	1770	4.15	0	69.5	296	1770	5.01	
0.007	21.0	48	380		0.042	57.3				
0.021	3.5	12	100		0.083	52.8				
0.042	0.2	0	12		0.167	43.9				
					0.250	39.9				
					0.333	35.4	190	1500		
					0.500	31.3	100	770		
					1.00	13.5	100	770	5.02	
					1.50	9.1	48	380		
					2.00	3.2	48	380		
					3.00	2.4	24	190		
					4.00	0.7	12	100	5.15	
					5.00		6	48		
					6.00		3	24	5.10	
V: 3.					V: 7.					
8.75 cc 0.1 n HCl		50°		15. 1. 24	2.5 cc 0.1 n HCl		50°		14. 12. 23	
0	69.5	296	1770	4.33	0	69.5	296	1770	5.82	
0.007	35.9	190	1500		0.042	62.7				
0.021	14.5	100	770		0.083	59.8				
0.042	5.2	48	380		0.167	59.4				
0.083	0.4	12	100		0.250	57.8				
0.125		3	24		0.333	56.1				
					0.500	53.5				
					1.00	41.4			5.87	
					1.50	36.0				
					2.00	25.1	190	1500		
					3.00	17.6	190	1500		
					4.00	10.7	100	770	5.93	
					5.00	6.4	100	770		
					6.00	4.2	48	380		
					7.04	3.0	24	190	5.99	
V: 4.										
7.5 cc 0.1 n HCl		50°		14. 1. 24						
0	69.5	296	1770	4.46						
0.007	52.2									
0.021	37.5									
0.042	22.7	190	1500							
0.083	11.2	100	770							
0.125	5.8	48	380							
0.167	3.9	48	380	4.47						
0.250	0.7	24	190							
0.333		6	48							
0.500		3	24							

V : 8.					V : 10.				
50° 12. 12. 23					5 cc 0.1 n NaOH 50° 13. 12. 23				
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>
0	69.5	296	1770		0	69.5	296	1770	
0.042	61.0				0.021	50.8			
0.083	59.6			6.60	0.042	37.6			
0.167	54.9				0.083	20.7			
0.250	53.6				0.167	9.1			7.67
0.333	51.9				0.250	8.3			
0.500	45.3				0.333	6.9			
1.00	27.5			6.56	0.500	6.2			
1.50	19.9	190	1500		1.00	5.0	190	1500	7.42
2.00	14.3	190	1500		1.50	4.3	190	1500	
3.00	7.7	100	770	6.53	2.00	3.8	190	1500	7.34
4.00	3.8	100	770		3.00	2.7	100	1500	
5.00	2.5	100	770		4.00	1.4	100	770	
6.00	1.1	48	380		5.00	1.0	48	770	
7.00		48	380		6.00		48	380	
8.00		24	190		7.00		24	190	
9.00		24	190	6.33	8.00		24	190	6.92

V : 9.					V : 11.				
2.5 cc 0.1 n NaOH 50° 8. 1. 24					7.5 cc 0.1 n NaOH 50° 18. 1. 24				
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>
0	69.5	296	1770		0	69.5	296	1770	
0.021	57.4				0.007	44.0			
0.042	54.6				0.021	15.6			
0.083	45.6				0.042	8.7			
0.167	31.7			7.05	0.083	6.3			7.93
0.250	27.9				0.167	6.0			
0.333	24.7				0.250	5.9			
0.500	17.6				0.333	5.7			
1.00	8.1			7.01	0.500	5.5			
1.50	6.2				1.00	5.2			7.76
2.00	5.1	190	1500		1.63	3.9	190	1500	
3.00	3.2	100	770		2.13	2.7	100	770	
4.00	2.5	100	770		3.00	1.7	100	770	
5.00	1.6	48	380		4.00	1.1	48	380	7.37
6.00	1.2	48	380	6.70	5.00		48	380	
7.00	0.6	24	190		6.00		24	190	
8.00		24	190		7.00		24	190	
9.00		12	100		8.00		12	100	
10.0		12	100		9.00		12	100	
11.0		6	48	6.32	10.0		12	100	
					11.0		6	48	6.77

V : 12.					V : 14.				
10 cc 0.1 n NaOH		50°	21. 1. 24		15 cc 0.1 n NaOH		50°	25. 1. 24	
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>
0	69.5	296	1770	8.51	0	69.5	296	1770	8.94
0.007	12.9				0.007	6.7			
0.021	7.8				0.021	6.3			
0.042	6.3			8.33	0.042	6.2			8.88
0.083	6.2				0.083	6.0			
0.167	6.0				0.167	5.7			
0.250	5.9	190	1500	8.24	0.250	5.4			8.62
0.333	5.9				0.333	4.9			
0.500	5.4				0.500	4.1	190	1500	
1.00	4.7	190	1500	7.61	1.00	3.1	100	770	7.87
1.50	2.8	100	770		2.00	1.8	100	770	
2.00	2.2	100	770		3.00	0.8	48	380	
3.00	1.7	48	380	7.24	4.00		24	190	7.45
4.00	1.1	48	380		5.00		24	190	
5.00		24	190		6.00		12	100	
6.00		24	190		7.00		12	100	
7.00		24	190		8.00		12	100	
8.00		12	100						

V : 13.					V : 15.				
12.5 cc 0.1 n NaOH		50°	24. 1. 24		20 cc 0.1 n NaOH		50°	4. 2. 24	
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>
0	69.5	296	1770	8.79	0	69.5	296	1770	9.38
0.007	7.3				0.007	6.1			
0.021	7.0				0.021	5.7			
0.042	6.5			8.62	0.042	5.3			9.30
0.083	6.2				0.083	4.9			
0.167	5.9				0.167	4.3			
0.250	5.7			8.37	0.250	3.5	190	1500	8.92
0.333	5.4				0.333	3.0	190	1500	
0.500	4.9	190	1500		0.500	2.6	100	770	
1.00	3.9	100	770	7.62	1.00	1.6	100	770	7.82
1.50	2.5	100	770		1.50	0.3	48	380	
2.00	1.7	100	770		2.00		48	380	
3.00	1.2	48	380	7.15	3.00		24	190	
4.00	0.4	48	380		4.00		12	100	
5.00		24	190						
6.00		24	190						
7.00		12	100						
8.00		12	100						
9.00		12	100						

V: 16.				
25 cc 0.1 n NaOH		50°	5. 2. 24	
days	maltose	X	Y	P <sub>H</sub>
0	69.5	296	1770	9.71
0.007	5.6			
0.021	5.4			
0.042	4.8			
0.083	4.0	190	1500	
0.167	3.0	100	770	9.59
0.250	1.1	100	770	
0.333	0.0	100	770	
0.500		48	380	
1.00		24	190	
1.50		12	100	8.62
2.00		6	48	

VI: 1.				
10 cc 0.1 n HCl		60°	17. 10. 23	
days	maltose	X	Y	P <sub>H</sub>
0	69.5	296	1770	4.23
0.021	0.0	0	12	

VI: 2.				
8.75 cc 0.1 n HCl		60°	18. 10. 23	
days	maltose	X	Y	P <sub>H</sub>
0	69.5	296	1770	4.38
0.021	1.0	48	380	
0.042	0.0	12	100	
0.083		0	6	

V: 17.				
30 cc 0.1 n NaOH		50°	6. 2. 24	
days	maltose	X	Y	P <sub>H</sub>
0	69.5	296	1770	9.97
0.007	4.3			
0.021	2.7	190	1500	
0.042	1.6	100	770	
0.083	0.3	48	380	
0.167		48	380	9.82
0.250		24	190	
0.333		12	100	
0.500		6	48	

VI: 3.				
7.5 cc 0.1 n HCl		60°	15. 10. 23	
days	maltose	X	Y	P <sub>H</sub>
0	69.5	296	1770	4.64
0.021	3.3	100	770	
0.042	2.3	48	380	
0.083	0.1	24	190	
0.125		12	100	
0.167		6	48	
0.208		3	24	

V: 18.				
35 cc 0.1 n NaOH		50°	7. 2. 24	
days	maltose	X	Y	P <sub>H</sub>
0	69.5	296	1770	10.27
0.007	2.8	100	770	
0.021	1.9	48	380	
0.042	0.0	24	190	
0.083		6	48	

VI: 4.				
6.25 cc 0.1 n HCl		60°	29. 10. 23	
days	maltose	X	Y	P <sub>H</sub>
0	69.5	296	1770	4.88
0.007	25.6			
0.021	5.1	190	1500	
0.042	3.5	100	770	
0.083	2.9	100	770	
0.125	1.5	48	380	
0.167	0.8	48	380	
0.250		24	190	
0.333		12	100	
0.417		6	48	

V: 19.				
40 cc 0.1 n NaOH		50°	8. 2. 24	
days	maltose	X	Y	P <sub>H</sub>
0	69.5	296	1770	10.50
0.007	0.8	24	190	
0.021		3	24	

VI: 5.					VI: 8.				
5 cc 0.1 n HCl 60° 19. 11. 23					2.5 cc 0.1 n NaOH 60° 3. 12. 23				
days	maltose	N	Y	p <sub>H</sub>	days	maltose	N	Y	p <sub>H</sub>
0	69.5	296	1770		0	69.5	256	1770	
0.007	40.5			5.10	0.007	11.8			7.18
0.021	13.2				0.021	7.8			
0.042	6.7	190	1500		0.042	7.1			
0.083	3.7	190	1500	5.06	0.083	6.0			7.16
0.125	2.9	100	770		0.125	5.4			
0.167	2.1	100	770		0.164	5.2			
0.250	1.3	48	380		0.250	5.1	190	1500	
0.333	1.0	48	380		0.333	3.2	100	770	
0.417	0.5	24	190		0.417	2.5	100	770	
1.00	0.0	3	24	5.20	0.500	2.2	48	380	
					1.00	0.0	12	100	6.92
					1.29		6	48	
					2.00		3	24	6.72
VI: 6.					VI: 9.				
2.5 cc 0.1 n HCl 60° 3. 12. 23					5 cc 0.1 n NaOH 60° 7. 11. 23				
0	69.5	296	1770		0	69.5	296	1770	
0.007	52.8			5.90	0.007	7.4			7.72
0.021	29.9				0.017	7.0			
0.042	16.5				0.042	6.2			
0.083	9.3			5.91	0.083	5.9			
0.125	6.4				0.134	5.8			
0.167	5.6				0.167	5.3	190	1500	
0.250	4.2	190	1500		0.250	3.1	190	1500	
0.333	3.4	100	770		0.333	2.1	100	770	
0.417	3.1	100	770		0.417	1.6	100	770	
0.500	2.1	100	770		0.500	1.2	48	380	
1.00	0.7	48	380	6.01	1.00	0.0	6	100	7.30
1.29	0.0	12	100						
2.00		3	24	6.17	VI: 10.				
					7.5 cc 0.1 n NaOH 60° 20. 11. 23				
0	69.5	296	1770		0	69.5	296	1770	
0.007	42.9			6.74	0.007	7.5			8.18
0.021	14.7				0.021	6.7			
0.042	8.3				0.042	6.1			
0.083	7.2				0.083	5.4			7.96
0.125	6.7				0.125	4.0	190	1500	
0.167	6.0	190	1500		0.167	3.0	100	770	
0.250	4.9	190	1500		0.250	1.9	100	770	
0.333	4.0	190	1500		0.333	1.1	48	380	
0.417	3.0	190	1500		0.417	0.6	24	380	
1.00	0.0	48	380		1.00	0.0	0	12	7.46
1.29		24	190						
2.00		3	24	6.28					
					VI: 7.				
60° 30. 10. 23									
0	69.5	296	1770						
0.007	42.9			6.74					
0.021	14.7								
0.042	8.3								
0.083	7.2								
0.125	6.7								
0.167	6.0	190	1500						
0.250	4.9	190	1500						
0.333	4.0	190	1500						
0.417	3.0	190	1500						
1.00	0.0	48	380						
1.29		24	190						
2.00		3	24	6.28					



## VI: 11.

10 cc 0.1 n NaOH 60° 23. 11. 23

days	maltose	N	Y	pH
0	69.5	296	1770	8.53
0.007	7.5			
0.021	6.3	190	1500	
0.042	5.0	190	1500	
0.083	4.3	100	770	8.25
0.125	2.8	100	770	
0.167	2.3	48	380	
0.250	0.7	48	380	
0.333	0.0	24	190	8.01
0.500		6	48	

## VI: 12.

12.5 cc 0.1 n NaOH 60° 6. 11. 23

days	maltose	N	Y	pH
0	69.5	296	1770	8.74
0.007	6.6	190	1500	
0.021	5.8	100	770	
0.042	4.7	100	770	
0.083	3.7	48	380	
0.125	1.8	48	380	
0.167	1.3	24	190	
0.250		24	190	
0.333		12	100	8.42

## VI: 13.

15 cc 0.1 n NaOH 60° 17. 11. 23

days	maltose	N	Y	pH
0	69.5	296	1770	9.15
0.007	6.6	190	1500	
0.021	5.0	100	770	
0.042	4.3	100	770	
0.083	2.0	48	380	9.09
0.125	0.0	24	190	
0.167		12	100	
0.250		0	12	

## VI: 14.

17.5 cc 0.1 n NaOH 60° 24. 11. 23

days	maltose	N	Y	pH
0	69.5	296	1770	9.21
0.007	5.6	190	1500	
0.021	4.0	100	770	
0.042	2.9	48	380	
0.083	0.5	24	190	9.22
0.140	0.0	6	48	
0.167		3	24	9.01

## VI: 15.

20 cc 0.1 n NaOH 60° 26. 11. 23

days	maltose	N	Y	pH
0	69.5	296	1770	9.43
0.007	5.1	100	770	
0.021	3.1	48	380	
0.042	1.3	24	190	
0.083	0.0	3	24	9.24

## VI: 16.

22.5 cc 0.1 n NaOH 60° 27. 11. 23

days	maltose	N	Y	pH
0	69.5	296	1770	9.61
0.007	5.7	100	770	
0.021	2.2	48	380	
0.042	0.0	12	100	
0.083		0	6	9.49

## VI: 17.

25 cc 0.1 n NaOH 60° 28. 11. 23

days	maltose	N	Y	pH
0	69.5	296	1770	9.73
0.007	4.3	100	770	
0.021	0.5	24	190	
0.042		0	12	
				9.67

## VI: 18.

27.5 cc 0.1 n NaOH 60° 29. 11. 23

days	maltose	N	Y	pH
0	69.5	296	1770	9.95
0.007	2.4	48	380	
0.021	0.0	3	24	

## VII: 1.

7.5 cc 0.1 n HCl 70° 27. 9. 23

days	maltose	X	Y	P <sub>H</sub>
0	72.5	296	1770	
0.007	0.8	24	190	
0.014	0.0	3	24	4.65

## VII: 2.

6.25 cc 0.1 n HCl 70° 23. 9. 23

days	maltose	X	Y	P <sub>H</sub>
0	72.5	296	1770	
0.007	3.3	100	770	
0.014	0.1	24	190	
0.021	0.0	12	100	
0.028		6	48	4.89

## VII: 3.

5 cc 0.1 n HCl 70° 21. 9. 23

days	maltose	X	Y	P <sub>H</sub>
0	72.5	296	1770	
0.007	4.0	100	770	
0.014	2.3	100	770	
0.021	1.0	48	380	
0.028	0.2	48	380	
0.042	0.0	24	190	
0.063		6	48	5.15

## VII: 4.

3.75 cc 0.1 n HCl 70° 19. 9. 23

days	maltose	X	Y	P <sub>H</sub>
0	72.5	296	1770	
0.007	4.7	190	1500	
0.014	3.5	100	770	
0.021	2.5	100	770	
0.028	1.6	100	770	
0.042	0.8	48	380	
0.063	0.0	48	380	
0.083		24	190	
0.125		6	48	
0.167		0	12	5.60

## VII: 5.

2.5 cc 0.1 n HCl 70° 17. 9. 23

days	maltose	X	Y	P <sub>H</sub>
0	72.5	296	1770	
0.007	6.3	190	1500	
0.014	4.7	190	1500	
0.021	4.0	190	1500	
0.028	2.7	190	1500	
0.042	1.8	100	770	
0.063	1.0	48	380	
0.083	0.2	24	190	
0.125	0.0	12	100	
0.167		6	48	6.12

## VII: 6.

70° 14. 9. 23

days	maltose	X	Y	P <sub>H</sub>
0	72.5	296	1770	
0.007	6.0	190	1500	
0.014	5.4	190	1500	
0.021	4.7			
0.028	4.1	190	1500	
0.042	3.6	100	770	
0.083	2.0	48	380	
0.125	0.9	24	190	
0.167	0.3	6	48	6.42

## VII: 7.

2.5 cc 0.1 n NaOH 70° 28. 9. 23

days	maltose	X	Y	P <sub>H</sub>
0	72.5	296	1770	
0.007	6.7	190	1500	
0.014	5.7	190	1500	
0.021	4.4	190	1500	
0.028	3.8	100	770	
0.042	2.2	100	770	
0.063	1.1	24	190	
0.083	0.8	12	100	
0.125	0.0	3	24	7.06

VII: 8.				
3.75 cc 0.1 n NaOH		70°	1. 10. 23	
days	maltose	N	V	pH
0	72.5	296	1770	
0.007	5.9	190	1500	
0.014	4.2	190	1500	
0.021	2.9	100	770	
0.028	2.1	100	770	
0.042	1.2	48	380	
0.063	0.4	12	100	
0.083		3	24	7.47

VII: 9.				
5 cc 0.1 n NaOH		70°	3. 10. 23	
0	72.5	296	1770	
0.007	5.3	190	1500	
0.014	3.2	190	1500	
0.021	2.0	100	770	
0.028	0.5	48	380	
0.042	0.0	12	100	
0.063		3	24	7.67

VII: 10.				
6.25 cc 0.1 n NaOH		70°	5. 10. 23	
0	72.5	296	1770	
0.007	5.3	190	1500	
0.014	2.2	100	770	
0.021	0.3	48	380	
0.028		24	190	
0.042		3	24	7.84

VII: 11.				
7.5 cc 0.1 n NaOH		70°	8. 10. 23	
0	72.5	296	1770	
0.007	4.3	190	1500	
0.014	2.1	48	380	
0.021	0.0	24	190	
0.028		3	24	8.22

VII: 12.				
8.75 cc 0.1 n NaOH		70°	8. 10. 23	
days	maltose	N	V	pH
0	72.5	296	1770	
0.007	3.8	190	1500	
0.014	1.1	24	190	
0.021		3	24	8.46

VII: 13.				
10 cc 0.1 n NaOH		70°	10. 10. 23	
0	72.5	296	1770	
0.007	3.0	100	770	
0.014	0.4	12	100	
0.021		0	12	8.67

VII: 14.				
11.25 cc 0.1 n NaOH		70°	10. 10. 23	
0	72.5	296	1770	
0.007	1.9	48	380	
0.014	0.0	6	48	
0.021		0	6	8.85

VIII: 1.				
5 cc 0.1 n HCl		75°	30. 8. 23	
0	72.5	296	1770	
0.007	1.1	24	190	
0.014	0.0	0	12	5.00

VIII: 2.				
3.75 cc 0.1 n HCl		75°	1. 9. 23	
0	72.5	296	1770	
0.007	1.6	100	770	
0.014	0.2	24	190	
0.021	0.0	12	100	
0.028		3	24	5.52

VIII: 3.					VIII: 7.				
2.5 cc 0.1 n HCl 75° 29. 8. 23					5 cc 0.1 n NaOH 75° 6. 9. 23				
days	maltose	X	Y	p <sub>H</sub>	days	maltose	X	Y	p <sub>H</sub>
0	72.5	296	1770		0	72.5	296	1770	
0.007	3.5	190	1500		0.007	1.5	48	380	
0.014	1.1	100	770		0.014	0.0	0	24	7.70
0.021	0.5	48	380						
0.028	0.0	24	190						
0.035		12	100						
0.042		6	48	6.05					
VIII: 4.					IX: 1.				
75° 27. 8. 23					2.5 cc 0.1 n HCl 80° 25. 8. 23				
0	72.5	296	1770		0	72.5	296	1770	
0.007	6.5	190	1500		0.003	2.1	48	380	
0.014	2.9	100	770		0.007	1.0	6	48	
0.021	1.6	48	380		0.010	0.4	0	6	5.93
0.028	0.8	24	190						
0.035	0.5	24	190						
0.042		12	100	6.52					
VIII: 5.					IX: 2.				
2.5 cc 0.1 n NaOH 75° 4. 9. 23					80° 23. 8. 23				
0	72.5	296	1770		0	72.5	296	1770	
0.007	3.0	100	770		0.003	5.5	190	1500	
0.014	1.3	24	380		0.007	2.6	100	770	
0.021	0.5	6	48		0.010	1.5	24	190	
0.028	0.0	0	12	7.04	0.014	0.2	6	48	6.66
VIII: 6.					IX: 3.				
3.75 cc 0.1 n NaOH 75° 5. 9. 23					2.5 cc 0.1 n NaOH 80° 24. 8. 23				
0	72.5	296	1770		0	72.5	296	1770	
0.007	2.3	100	770		0.003	2.1	100	770	
0.014	0.2	12	100		0.007	0.0	3	24	
0.021	0.0	0	12	7.44	0.010		0	3	7.15

With increase of temperature, the stability of the enzyme decreases, but an increase of temperature affects the saccharogenamylase more than it does the dextrinogenamylase. At each temperature, the concentration of hydrogen ions is of the same importance as before, the stability being greatest at a certain value of  $p_H$ , but less on either side of this. This is shown in figs. 1—7. As a measure of the stability of the enzyme, we have here taken the time required to halve it; i. e. the time it takes for the values given in the tables under »maltose« and »X« to fall from a certain figure to another half its magnitude. The halving time is in this connection a very loose term, the employment of which is really altogether unjustified, but it enables us to show, in a few plain curves, the main results of the tables given in the foregoing. The actual halving time cannot be calculated quite exactly, but only estimated with a certain arbitrary approximation. For the use of it presupposes that the process follows the laws for a simple, monomolecular reaction, and this is by no means the case here. Moreover, it postulates a simple proportionality between the quantities of »maltose« and the quantity of enzyme, which actually only exists within certain limits. We should not, therefore, attribute further significance to these curves beyond the fact that they enable us to see at a glance the approximate general outline of the results obtained.

It will be seen that a rise of temperature greatly alters the height of the curves, i. e. stability of the enzymes<sup>1)</sup> but has hardly any effect on the position of the curves relative to the abscissa, i. e. on the influence of hydrogen ion concentration upon the stability. Saccharogenamylase for instance, has its maximum stability at abt.  $p_H = 6$ , and dextrinogenamylase at abt.  $p_H = 6.5$ , irrespective of the temperature.

At ice box temperature, the curve for saccharogenamylase lies considerably above that for dextrinogenamylase, save in the most alkaline solutions. The curves intersect at abt.  $p_H = 8.5$ , after which that of the dextrinogenamylase lies just a little above that of the saccharogenamylase. At  $38^\circ$ , the curves are of about the same height, but the one for saccharogenamylase is here, as always, slightly displaced in the direction of lower  $p_H$  as compared with the curve for dextrinogenamylase. At  $50^\circ$ , the curve for saccharogenamylase is already so low as compared with that

<sup>1)</sup> The scale of height is varied in the different figures.

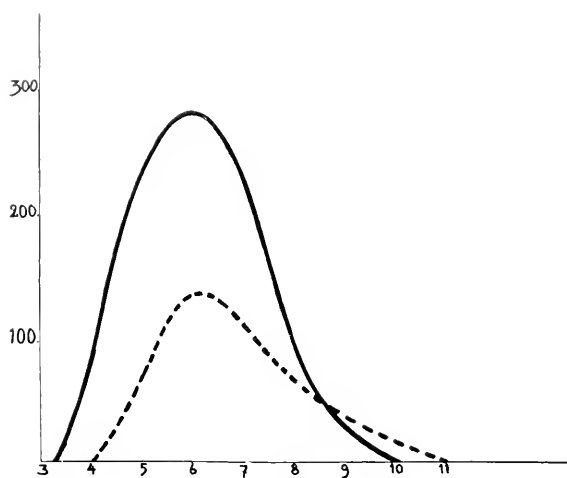


Fig. 1.

Halving times at abt. 60°.

— saccharogenamylase.  
- - - dextrinogenamylase.

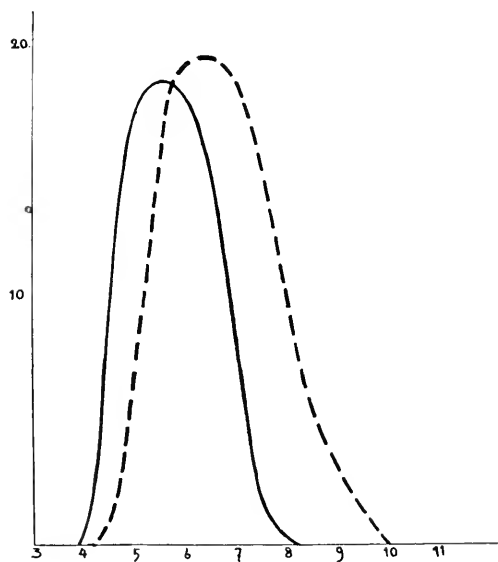


Fig. 2.

Halving times at 38°.

— saccharogenamylase.  
- - - dextrinogenamylase.

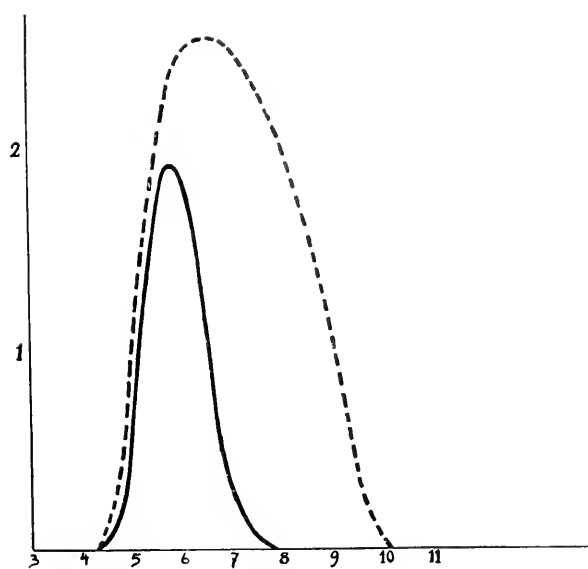


Fig. 3.

Halving times at 50°.

— saccharogenamylase.  
 - - - dextrinogenamylase.

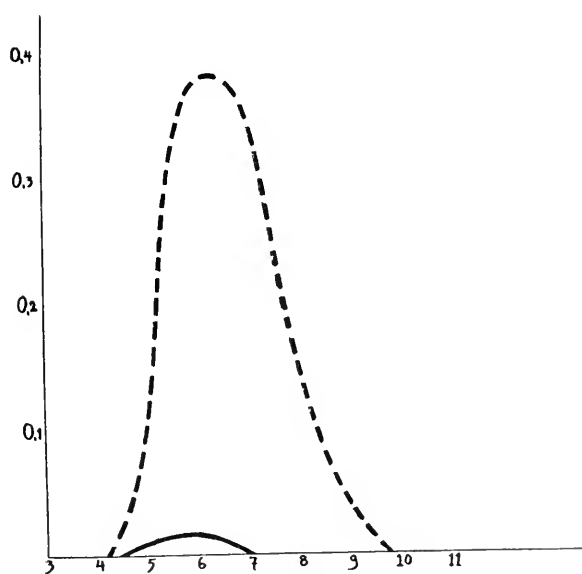


Fig. 4.

Halving times at 60°.

— saccharogenamylase.  
 - - - dextrinogenamylase.

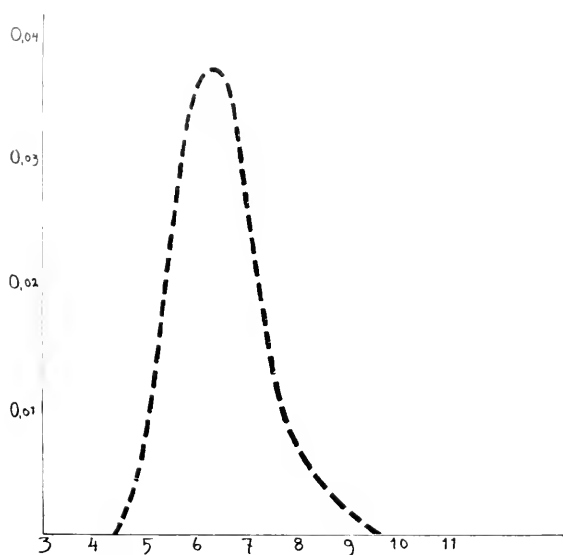


Fig. 5.

Halving times for dextrinogenamylase at 70°.

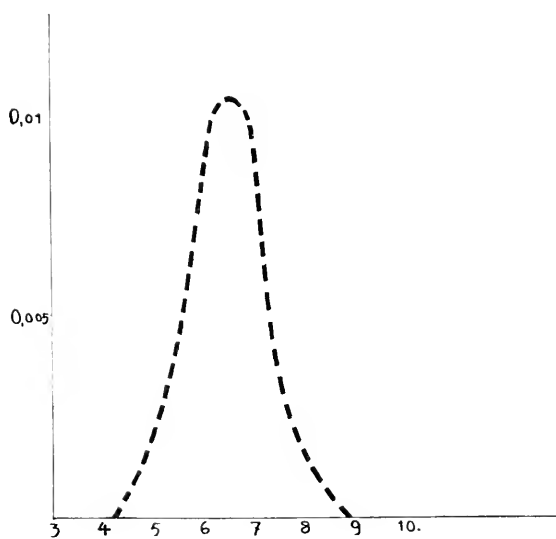


Fig. 6.

Halving times for dextrinogenamylase at 75°.



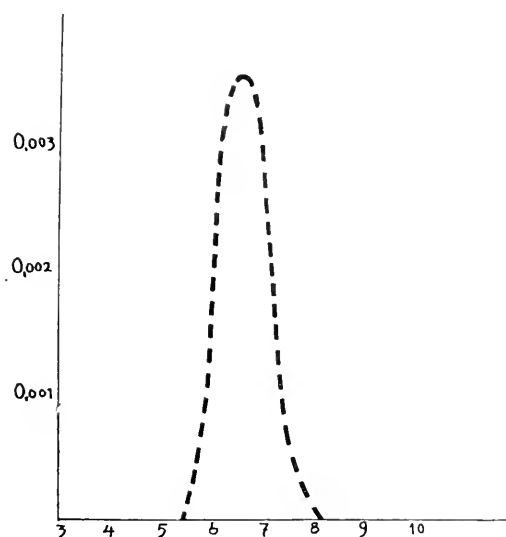


Fig. 7.  
Halving times for dextrinogenamylase at 50°.

for dextrinogenamylase that it lies below this throughout. At 60°, the curve for saccharogenamylase is reduced to insignificance compared with that for dextrinogenamylase, which is still further accentuated at higher temperature again.

Taking for instance the experiments in Series VII, we find that the maltose values after a lapse of only 0.007 days — i. e. 10 minutes — have fallen from 72.5 to 6.7 mg or less, after which their further decrease proceeds very slowly indeed. This last slow decrease proceeds entirely parallel with the reduction in the quantities X and Y. This, it seems to me, can only be explained by supposing that the slight, but long retained power of forming »maltose« or at any rate, reducing substances, does not depend on a last remainder of the saccharogenamylase, but is a function of the dextrinogenamylase. This question is further discussed in Chap. 9.

## 6. Isolation of the two enzymes one from another.

The investigations above described show that it is possible, at any rate to a certain extent, to destroy either the dextrinogenamylase or the saccharogenamylase under such conditions that the other enzyme is only slightly altered. We thus obtain solutions of the respective enzymes, each more or less free from any

admixture of the other. These will in the following be termed saccharogenamylase solution and dextrinogenamylase solution. The experiments noted above also afford the requisite information for preparation of these solutions. We can here combine temperature, hydrogen ion concentration and time in many different ways, and still arrive at more or less the same result. And it will therefore perhaps be advisable to choose such temperatures and concentrations of hydrogen ions, as permit of the experiment being concluded in a short time.

#### 6 A. Preparation of saccharogenamylase.

Owing to the greater sensitiveness of saccharogenamylase to heat, it must always be prepared at a low temperature. The experimental series I, II and III show that saccharogenamylase at  $p_H = 3-4$  is quite stable, whereas dextrinogenamylase is highly unstable at this concentration of hydrogen ions and at ice box temperature. In order to arrive at a method of preparing saccharogenamylase on these lines, the following experiments have been made.

To 50 cc malt solution is added a certain quantity of 0.1 n hydrochloric acid, and sufficient water to bring the volume up to 100 cc. After the lapse of 15 minutes, as much  $m/15$  solution of secondary phosphate, and as much water, are added as to make the total volume 200 cc, and  $p_H$  abt. 6, at which concentration of hydrogen ions both enzymes are very stable. All the liquids are cooled with ice water before commencing the experiment, and the flask is kept immersed in ice water throughout the whole of the experiment. Table 5 shows the result of two series of experiments, made with different malt solutions. The table gives first the amount of hydrochloric acid used, and the concentration of hydrogen ions thus produced, then the quantity of secondary sodium phosphate employed and the hydrogen ion concentration thereby arrived at, with finally, the values for maltose and X arrived at in the usual manner. The last values in each series of experiments refer to the original, suitably diluted malt solution <sup>1)</sup>.

By adding to a malt solution at 0° a sufficient quantity of 0.1 n hydrochloric acid to give  $p_H =$  abt. 3.3, and interrupting the experiment after 15 minutes by adding sufficient secondary

<sup>1)</sup>  $p_H$  determined with the quinhydrone electrode.

Table 5.

cc HCl	p <sub>H</sub>	cc phos- phate	p <sub>H</sub>	maltose	N	cc HCl	p <sub>H</sub>	cc phos- phate	p <sub>H</sub>	maltose	N
40	2.81	72	5.65	4.2	0	40	2.77	72	6.02	1.8	0
35	3.08	63	5.67	22.0	3	35	2.96	63	6.05	13.1	3
30	3.23	54	5.52	26.4	3	30	3.20	54	6.03	25.7	3
25	3.39	45	5.46	28.6	6	25	3.48	45	6.03	27.8	6
22.5	3.49	40.5	5.46	28.3	6	22.5	3.60	40.5	6.06	28.0	6
20	3.64	36	5.45	28.5	24	20	3.79	36	6.08	27.8	48
17.5	3.76	31.5	5.34	32.2	100	17.5	3.94	31.5	6.05	28.7	100
15	3.88	27	5.32	32.9	100	15	4.10	27	6.08	29.8	100
—	—	—	5.35	37.4	380	—	—	—	5.86	30.6	380

sodium phosphate to give  $p_H = \text{abt. } 6$ , we thus obtain a powerfully active saccharogenamylase solution, with an activity of 70—80 % of that of the original solution, while the activity of the dextrinogenamylase has fallen to 1—2 % of the original value.

#### 6 B. Preparation of dextrinogenamylase.

It is possible to prepare a solution of dextrinogenamylase in a similar manner to that used for the saccharogenamylase, by making the reaction alkaline at low temperature. Here, however, the relation between the stabilities of the two enzymes is less favourable, for in order to effect sufficiently rapid destruction of the saccharogenamylase, it is necessary to render the solution so highly alkaline, that the dextrinogenamylase itself is also destroyed to an essential degree. It will be immediately apparent from figs. 1—7 that better results may be looked for at higher temperatures, and the experimental series IV—IX give all the requisite data as to this.

The preparation may be carried out as follows. In a water thermostat at  $70^\circ$ , 75 cc of water is heated in a flask to the same temperature. 25 cc malt solution is then added, and the flask is held immersed in water and gently shaken. After then minutes, the flask is cooled in cold water. During the process of heating, a precipitate of coagulated protein is formed; this however, does not affect the activity of the enzyme, and it is immaterial whether the precipitate is filtered off or allowed to remain. Dilution with water does not of course form an essen-

tial item in the method of preparation, but an undiluted malt solution should, to be on the safe side, be heated somewhat longer, say 15 minutes.

Exp. VII:6, shows how the enzyme is altered by this mode of treatment. The sugar-forming capacity falls from 72.5 mg to 6.0, and even this small remainder may perhaps be a function of the dextrinogenamylase, and not of the saccharogenamylase, cf. Chap. 9. The dextrinogenamylase is relatively stable, falling from  $X = 296$  to  $X = 190$ , though in actual fact, the stability is perhaps higher than indicated by these figures. As already mentioned in Chap. 2, these values are subject to considerable inaccuracy owing to the method itself. This applies more especially to the value 190, which was obtained in accordance with »Series A«. Exp. VII:6 also shows that further heating for more than 30 minutes is required in order to bring down the value of  $X$  from 190 to its next stage, 100. That the dextrinogenamylase is in reality but very slightly affected in the preparation of dextrinogenamylase solution by the method above described will be seen from the following experiment, made with a different malt solution, having a concentration of hydrogen ions of  $p_H = 6.11$ . The activity of the saccharogenamylase fell from 33.0 mg to 2.7, while that of the dextrinogenamylase fell only from  $X = 159$  to  $X = 124$ .

In preparing a dextrinogenamylase solution according to this method, the concentration of hydrogen ions should be kept somewhere between  $p_H = 6$  and  $p_H = 7$ , as will be seen from the experiments in Series VII.

## **7. Influence of the concentration of hydrogen ions on the activity of saccharogenamylase.**

The experiments were carried out in the manner described in Chap. 2. The buffer solutions up to  $p_H = 8$  were composed of  $1/15$  molar solutions of phosphoric acid, primary potassium phosphate and secondary sodium phosphate, mixed in different proportions. The total content of phosphoric acid was thus kept constant. To obtain a  $p_H$  higher than 8, mixtures of sodium hydroxide and secondary phosphate must be used. Only small quantities of sodium hydroxide are required; the greatest quantity employed was 0.6 cc 0.1 n NaOH to 9.4 cc secondary phosphate, which gave  $p_H = 9.13$ .

Table 6.

P <sub>H</sub>	maltose		P <sub>H</sub>	maltose		P <sub>H</sub>	maltose	
	mg	%		mg	%		mg	%
2.72	0.0	0.0	4.94	30.9	96.0	7.21	26.0	80.7
2.86	0.9	2.8	5.52	31.9	99.1	7.80	21.5	66.8
3.14	9.7	30.1	5.70	30.1	93.5	8.21	14.2	44.1
3.35	22.5	69.9	5.77	29.3	91.0	8.32	11.8	36.6
3.69	27.0	83.9	6.06	28.0	87.0	8.36	6.2	19.3
4.13	31.6	98.1	6.52	26.7	82.9	9.13	0.3	0.9
4.67	32.2	100.0						

Table 7.

P <sub>H</sub>	maltose		P <sub>H</sub>	maltose		P <sub>H</sub>	maltose	
	mg	%		mg	%		mg	%
2.68	0.6	1.3	4.21	44.5	95.7	6.76	37.5	80.6
2.77	2.1	4.5	4.82	46.5	100.0	7.09	36.4	78.3
2.83	4.5	9.7	5.25	46.0	98.9	7.17	35.0	75.3
2.96	12.5	26.9	5.64	45.6	98.0	7.46	34.1	73.3
3.06	23.1	49.7	5.75	44.4	95.5	7.73	28.7	61.7
3.30	32.4	69.7	5.88	44.0	94.6	7.86	26.6	57.2
3.41	36.5	78.5	5.99	43.3	93.1	8.13	19.1	41.1
3.59	39.6	85.1	6.14	42.6	91.6	8.29	16.7	35.9
3.85	41.6	89.4	6.34	41.4	89.0	8.44	14.3	30.7
4.00	44.5	95.7	6.49	40.3	86.6	8.75	5.4	11.6

The results of the experiments are shown in Tables 6 and 7, and in Fig. 8. The tables give the hydrogen ion concentration as measured with the quinhydrone electrode, mg. maltose, and, in the last column, the quantity of maltose expressed in percentage of the highest value in the table. The experiments in Table 6 were made with a saccharogenamylase solution prepared in accordance with the method described in Chap. 6 A. This solution exhibited at the same time a dextrinogenamylase activity of  $X = 3$  and  $Y = 100$ . The experiments in Table 7 were made with the original malt solution from which the above-mentioned saccharogenamylase solution was prepared. This malt solution was diluted to four times its volume in order to render it fully

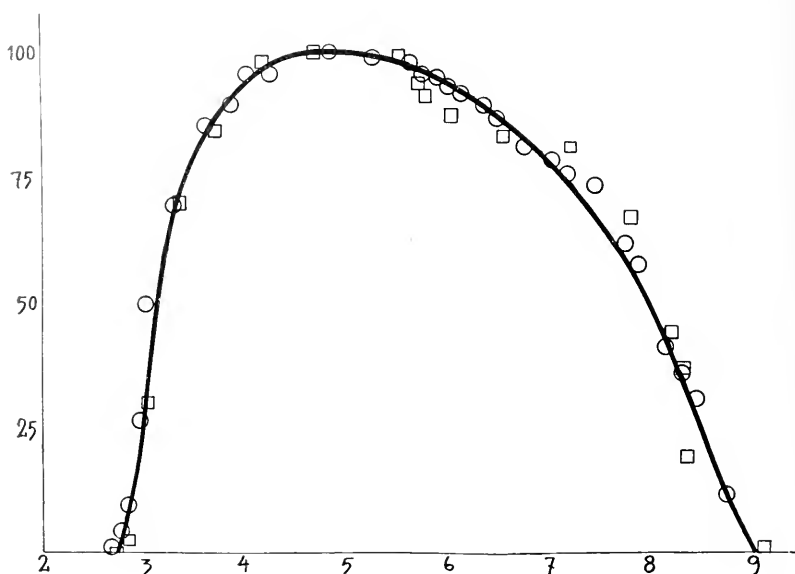


Fig. 8.

Activity of saccharogenamylase at different concentration of hydrogen ions.

□ table 6.

○ table 7.

comparable with the saccharogenamylase solution, in the preparation of which a similar dilution takes place. The malt solution thus diluted showed a dextrinogenamylase activity of  $X = 159$  and  $Y = 729$ .

Fig. 8 shows the percentages from the last column in both tables compared, and it will be noted that there is a very nice agreement between the two series of experiments. This suggests that the saccharogenamylase is not essentially altered in character by exposure to the highly acid reaction involved by preparation of the saccharogenamylase solution, whereas the dextrinogenamylase is brought down from  $X = 159$  to  $X = 3$ . The  $Y$  value cannot be taken in this connection as any measure of the activity of the dextrinogenamylase, as the high value of  $Y = 100$  is due, as explained in Chap. 9, in all probability not to the dextrinogenamylase but to the saccharogenamylase.

The optimum for saccharogenamylase lies, in both experiments, between  $p_H = 4$  and  $p_H = 5.75$ ; that is to say, we have an optimal zone of some considerable extent, not a single optimal point. The result agrees fairly well with the statements of previous investigators, which, like Table 7, refer to original malt

solution. There are however, certain points of difference. Adler<sup>1)</sup> used mainly acetate buffer, and has only a few experiments with phosphate buffer in the neighbourhood of optimum. His experiments were carried out at 20°. The values on the acid side of the optimum agree fairly well with mine, but on the alkaline side, he finds a considerably steeper fall, so that the activity at  $p_H = 7.25$  is already only 4.8 % of the maximum. Ernström<sup>2)</sup> has only 4 values obtained at 37° and with acetate buffer. He also finds a fairly broad optimal zone, between  $p_H = 4$  and  $p_H = 6$ . Three of his experiments lie within this range. The fourth, at  $p_H = 3.19$ , only shows an activity of 4 % of the maximum. The values which best agree with mine appear to be those of Sjöberg and Ericsson<sup>3)</sup> but even in comparison with these, the left portion of my curve seems to lie somewhat more toward the acid side.

### 8. Influence of the concentration of hydrogen ions on the activity of dextrinogenamylase.

The experiments were carried out in the usual way according to the method indicated in Chapt. 2, and with the same kind of buffer solutions as in the case of the saccharogenamylase. The concentration of hydrogen ions in the enzyme solution itself was abt.  $p_H = 6$ . If now an experiment be made at a relatively high acid reaction, say  $p_H = 4$ , the phosphates will, at this concentration of hydrogen ions, constitute a very slight buffer. The concentration of hydrogen ions is therefore determined partly from the enzyme solution, which itself contains phosphates separated from the malt. As the activity of the enzyme at this concentration of hydrogen ions is slight, it requires relatively large quantities of enzyme solution to hydrolyse the starch into products not stained by iodine. At the worst, it might happen that no colouring appeared in the tube containing 1 cc enzyme solution, but violet in that containing 0.5 cc. The contents of these tubes thus differ by no less than 0.5 cc enzym solution, and this may render the hydrogen ion concentration in the two tubes entirely different. It is therefore necessary, in all doubtful cases,

<sup>1)</sup> Biochem. Zeitschr. **77**, 146 (1916).

<sup>2)</sup> Zeitschr. f. physiol. Chem. **119**, 190 (1922).

<sup>3)</sup> Ibid. **139**, 118 (1924).

Table 8.

$p_H$	X	% X	Y	% Y	$p_H$	X	% X	Y	% Y
3.80	12	10	100	14	6.39	74	60	442	61
4.14	48	39	380	52	6.78	48	39	380	52
4.28	74	60	442	61	7.13	48	39	380	52
4.94	96	77	442	61	7.50	24	19	380	52
5.45	124	100	729	100	7.75	12	10	190	26
5.78	124	100	729	100	8.06	6	5	48	7
6.08	74	60	442	61					

Table 9.

$p_H$	X	%	$p_H$	X	%
3.54	3	2	6.02	159	100
3.92	6	4	6.37	124	78
3.99	12	8	6.67	74	47
4.21	48	30	7.12	57	36
4.53	96	60	7.45	24	15
4.69	124	78	7.75	24	15
5.34	124	78	7.95	24	15
5.72	159	100	8.10	12	8

to determine the concentration of hydrogen ions in both tubes. By slightly varying the composition of the buffer solutions in the different tubes, I have endeavoured to get the concentration of hydrogen ions in all tubes in one experiment as nearly alike as possible. In experiments lying within a range where the phosphate mixtures had strong buffer action, or where the tubes only contained small quantities of enzyme solution, the concentration of hydrogen ions was only determined in the last unstained tube.

Two series of experiments with dextrinogenamylase solution prepared as described in Chap. 6 B are shown in Tables 8 and 9, and fig. 9. The tables contain the values for  $p_H$  and X, Table 8 including also values for Y. Furthermore, X and Y are also stated in percentages of the highest value. The X percentage constitutes the ordinate in Fig. 9. The hydrogen ion concentration in Table 8 is measured with the quinhydrone electrode, in



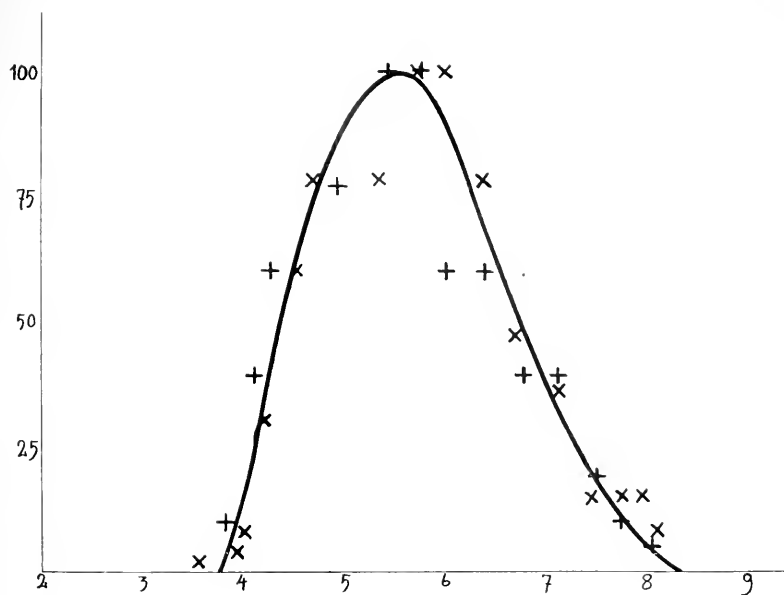


Fig. 9.

Activity of dextrinogenamylase at different concentrations of hydrogen ions.

+ table 8.

x table 9.

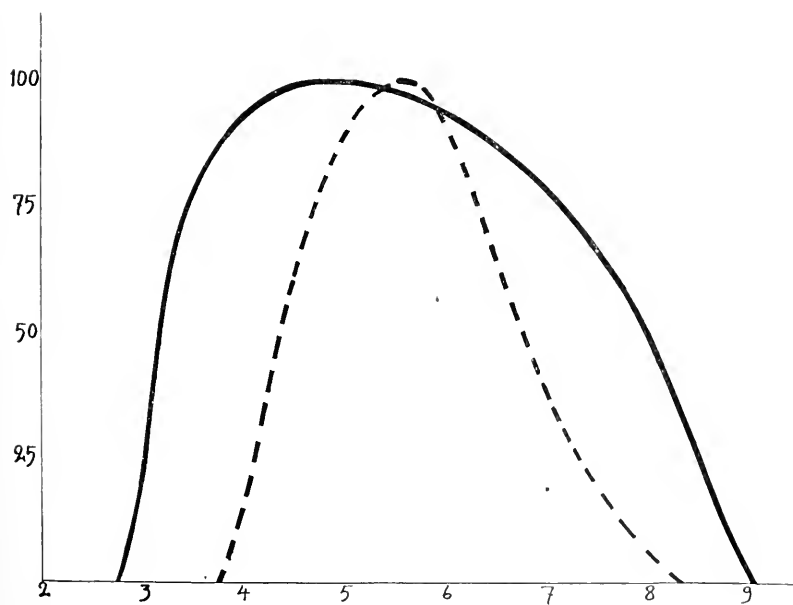


Fig. 10.

Activity of the enzymes at different concentrations of hydrogen ions.

— saccharogenamylase.

- - - dextrinogenamylase.

Table 10.

$p_H(X)$	$p_H(Y)$	X	% X	Y	% Y
3.92		12	6	100	7
4.18		48	25	380	25
4.62		100	53	770	51
5.13		190	100	1500	100
5.37		190	100	1500	100
5.71		100	53	770	51
6.00		100	53	770	51
6.20	6.24	48	25	770	51
6.45	6.73	6	3	380	25
6.66	7.08	3	2	380	25
6.87	7.46	3	2	190	13
7.48	7.77	0	0	100	7
7.72	8.12	0	0	48	3

Table 9 with hydrogen gas electrode. The enzyme solution employed in Table 8 had a saccharogenamylase activity of 2.7 mg maltose and in Table 9, 3.1 mg.

The agreement between the two series of experiments is certainly not particularly good, but we could hardly look for anything better when we consider the uncertainty inevitably attaching to determinations of this sort.

On comparing the activity curve of the dextrinogenamylase with that of the saccharogenamylase, Fig. 10, we find a considerable difference, a much greater than could arise out of the uncertainty in determination of the dextrinogenamylase. Dextrinogenamylase has not, like saccharogenamylase, a broad optimal zone, but rather an optimal point, situated between  $p_H = 5.5$  and 6.0. On either side of this, the curve runs more or less parallel with that for saccharogenamylase, but always inside this, at a distance of abt. 1 unit in  $p_H$ .

A corresponding investigation carried out with unaltered malt solution diluted in the proportion of 1:4, gave a quite unexpected result. A series of experiments of this nature is shown in Table 10 and Fig. 11. The determinations were made by the less accurate method, Series A. The concentration of hydrogen ions was measured with the quinhydrone electrode. The table shows

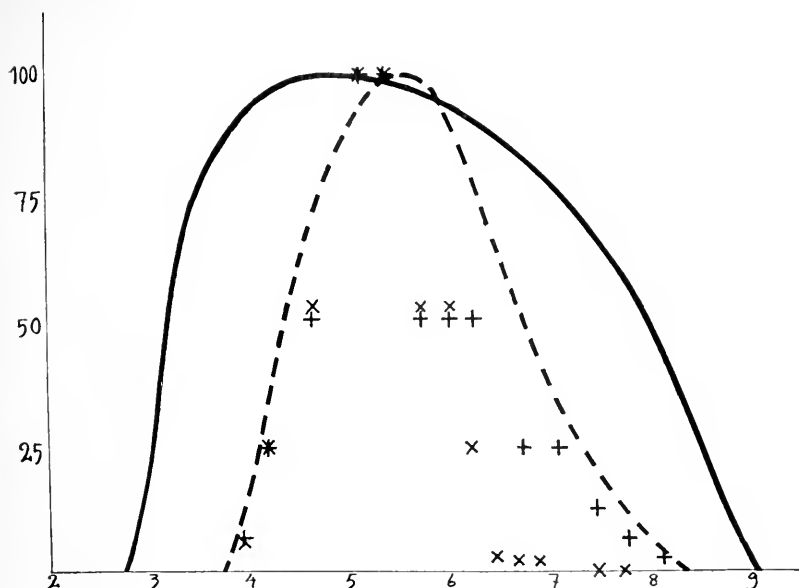


Fig. 11.

Activity of dextrinogenamylase in unaltered malt solution.

 $\times = X$ . $+ = Y$ .

the concentration of hydrogen ions in the last unstained tube,  $p_H(X)$ , and in some cases also the concentration of hydrogen ions in the last violet tube,  $p_H(Y)$ . It should be noted that these two figures appear essentially different in the 5 last experiments, the quantity of enzyme in the two tubes mentioned being very dissimilar. A double determination on these lines would have been desirable in the first experiment of the table also, but is unnecessary in the others. The table also shows  $X$  and  $Y$  and the values of these expressed in percentages of the highest value. With increasing  $p_H$ ,  $X$  and  $Y$  increase simultaneously and in the same proportion, reaching their maxima between  $p_H = 5.13$  and  $5.37$ , i. e. in fairly good agreement with the results already arrived at for dextrinogenamylase. On the other side of the maximum, however, the values fall more rapidly than for dextrinogenamylase solution, and from  $p_H = 6.2$  inclusive, the agreement between  $X$  and  $Y$  ceases to exist,  $Y$  keeping more or less to the values already noted for dextrinogenamylase, while  $X$  falls rapidly to very low values.

The malt solution here investigated was the same from which the dextrinogenamylase solution was made for use in the

experiments noted in Table 8. We might expect that the dextrinogenamylase should also be destroyed to some extent by heating, and that we should consequently find, on the whole, somewhat lower values for X and Y in Table 8 than in Table 10, though the degree of dilution is the same. This, however, is by no means the case. The first X values, on the acid side up to and a little beyond the optimum, are, it is true, alike in both tables, or slightly higher in Table 10, but as a concentration of hydrogen ions between  $p_H = 6.0$  and  $6.5$ , the position is reversed. In all the subsequent experiments, the heated enzyme exhibits a more powerful action than the non-heated. In the Y values, this phenomenon is only faintly indicated. For it is only the values at  $p_H = 7.5$  and  $7.75$  which are twice as great in Table 8 as in Table 10.

In order to explain this remarkable phenomenon, we must assume that the salt solution contains some substance which has an inhibiting effect on the dextrinogenamylase. Heating destroys this substance. The inhibition depends on the concentration of hydrogen ions.

This hypothesis is proved correct by the following experiment, which at the same time shows the resistance of the inhibiting substance to alkaline reaction. To 20 cc of malt solution is added 40 cc 0.1 n caustic soda and the whole placed in a thermostat at  $38^\circ$ . After 70 minutes, 60 cc water and 40 cc 0.1 n hydrochloric acid are added. A slight deposit has formed, and this is filtered off. By this mode of treatment, the dextrinogenamylase is completely destroyed. And as the saccharogenamylase, under these conditions, is more unstable than the dextrinogenamylase, it must likewise have been destroyed. The solution thus obtained is called in the following the inhibiting solution. In preparing the inhibiting solution, the original malt solution was diluted in the proportion of 1:8. The dextrinogenamylase solution as well as the enzyme solution in Table 10 contained the original malt solution in a dilution of 1:4. Thus 1 cc of the inhibition solution answers to 0.5 cc of either of these enzyme solutions.

The inhibiting solution contains, in addition to the elements derived from the malt solution, also sodium chloride at a concentration of 0.025 mol. per litre. In making the experiment

therefore, a 0.025 molar sodium chloride solution should be used for control purposes.

The experiment is carried out as follows: For the enzyme, a dextrinogenamylase solution is used, its activity being tested at different concentrations of hydrogen ions as shown in Table 10. The results are noted in Tables 11 and 12, and in Fig. 12. In the experiments in Table 11, each tube contains, besides its usual contents, 1 cc 0.026 molar sodium chloride solution; in Table 12 on the other hand, 1 cc of the inhibiting solution. The volume will thus here be 4 cc in each tube, whereas in all the other experiments it is 3 cc.

Table 11.

P <sub>H</sub>	X	% X	Y	% Y	P <sub>H</sub>	X	% X	Y	% Y
4.05	12	12	100	13	6.39	48	48	380	49
4.31	48	48	380	49	6.77	48	48	380	49
5.08	100	100	770	100	7.11	24	24	190	25
5.40	100	100	770	100	7.46	24	24	190	25
5.80	100	100	770	100	7.87	12	12	100	13
6.09	100	100	770	100	8.15	6	6	48	6

Table 12.

P <sub>H</sub>	X	% X	Y	% Y	P <sub>H</sub>	X	% X	Y	% Y
4.44	12	12	100	13	6.30	12	12	100	13
4.83	48	48	380	49	6.71	6	6	48	6
5.04	100	100	770	100	6.97	3	3	24	3
5.26	100	100	770	100	7.25	3	3	24	3
5.57	100	100	770	100	7.42	3	3	24	3
5.86	48	48	380	49	7.54	0	0	12	2
6.10	12	12	190	25					

Table 11 agrees with the results previously obtained with dextrinogenamylase solution, Tables 8 and 9. True, the agreement is not complete, but the differences can be fully and entirely explained by the fact that Tables 8 and 9 were based on the more exact method, series B, whereas the experiments in Tables 11 and 12 were made with series A, cf. Chap. 2. The

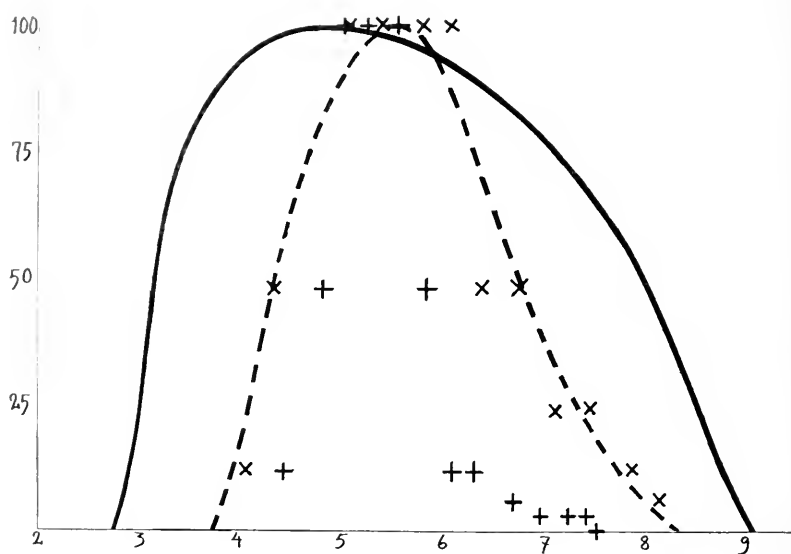


Fig. 12.

Activity of the dextrinogenamylase in the presence of inhibiting substance + and without same X.

presence of sodium chloride and dilution to 4 cc instead of 3 cc are thus of no importance.

In Table 12, we find, at  $p_H = 4.4$ , a lower activity than in Table 11; at  $p_H = 5.0$  and  $5.6$ , the same activity in both tables, but then the activity decreases much more rapidly in Table 12 than in Table 11. This confirms the results found in Table 10, and the explanation there given. In the preparation of the inhibiting solution, the enzymes have been destroyed by the strong alkaline reaction, but the inhibitor remains to a certain extent. In the experiments Table 12, the concentration of the inhibiting substance is the same in all tubes, as it is introduced only with the inhibiting solution, the dextrinogenamylase solution employed being free from this. We may therefore expect to find, at a certain concentration of hydrogen ions, the same inhibiting effect in all tubes, i. e. the same in the Y values as in the X values. This is also the case in Table 12, whereas in Table 10, Y falls considerably more slowly than X. This is evidently due to the fact that in the experiments in Table 10, the enzyme and the inhibitor are introduced in the same solution, viz. the unaltered malt solution. Just as the quantity of enzyme

in any tube is half that in the one before it, so also the amount of inhibitor in any tube is half that in the one before it. In reducing the quantity of enzyme in a series of tubes, the quantity of inhibitor is reduced at the same time, and thus also the inhibiting effect. This is less noticeable, the smaller the amount of enzyme; and as the quantity of enzyme is always smaller in the tubes which determine the Y value than in those for the X, it follows that Y must be less affected than X, as will also be seen to be the case in Table 10. In Table 12 on the other hand, the inhibitor is the same in all tubes, and X and Y are therefore inhibited in the same proportion.

As regards the nature of the inhibitor itself, it seems to me most natural to think first of the proteins. In the preparation of the dextrinogenamylase solution, the heating occasions a coagulation of the proteins, which are thrown off in a flaky precipitate. A dextrinogenamylase solution contained 0.65 g nitrogen per litre where the original malt solution contained 0.74 g per litre. The difference, 0.09 g, represents the coagulated proteins. Simultaneously with this coagulation, the inhibitor is also destroyed. If, on the other hand, we destroy the enzymes by exposing them to strong alkaline reaction, we obtain only a very slight precipitate in the form of a slight cloudiness; the proteins remain in the solution, which retains its inhibiting activity.

The influence of concentration of hydrogen ions on the inhibiting effect can be explained in more than one way. The following explanation seems to me likely enough, though proof is lacking. The enzymatic starch hydrolysis is preceded by some kind of union between starch and enzyme. The inhibitor also possesses the power of binding the enzyme to itself, and the enzyme is thus divided between the starch and the retarding substance, arriving at a state of equilibrium just as does a base distributing itself between two acids according to their avidity. The concentration of hydrogen ions affects the state of the proteins in the solution, and thus also their enzyme-binding capacity. The inhibiting effect has a minimum between  $p_{H} = 5$  and 5.5. At this concentration of hydrogen ions, then, the equilibrium should be most favourable for the union of enzyme-substrate.

### 9. Purity of the Enzyme solutions.

It has already been pointed out, (p. 41) as likely that the dextrinogenamylase also has the power of forming sugar, or at any rate reducing substances, even though this power may be considerably less than that of the saccharogenamylase. It is also likely, or indeed, obvious, that the saccharogenamylase must, to a certain extent, have the same effect as the dextrinogenamylase, though quantitatively less. For when the saccharogenamylase affects the starch in forming sugar, the starch of course disappears, and therewith also the susceptibility of the solution to staining with iodine.

In the experiments noted in Chap. 5, where the saccharogenamylase was destroyed more rapidly than the dextrinogenamylase, e. g. Exp. VII: 5, it is noticeable that the maltose values fall very rapidly at first, but later decrease parallel with the X and Y values, and disappear simultaneously with these. At a point where, from the initial velocity of the decomposition, we should expect that all saccharogenamylase must long since have been destroyed, there still remains the power of forming some few milligrammes of sugar, or a reducing substance corresponding to some few mg sugar. It is possible then to suppose that this does not depend on a small amount of the saccharogenamylase remaining, but that this sugar formation is due to the activity of the dextrinogenamylase. The parallelism between the maltose values on the one hand and the X and Y values on the other then becomes a matter of natural necessity. But from this it would also follow that the solutions of dextrinogenamylase prepared in accordance with Chap. 6B must be practically pure, i. e. free from saccharogenamylase, despite the fact that they still retain a certain power of forming reducing substance.

As the activity of the two enzymes varies in different ways with the concentration of hydrogen ions, we may expect to find experimental proof of the purity of the dextrinogenamylase solutions by investigating their capacity for sugar formation at different concentrations of hydrogen ions. Unfortunately, experiments in this direction gave no clear and definite result. A series is given in Table 13, showing the values found for hydrogen ion concentration (quinhydrone) and amount of maltose formed, expressed in mg and also in % of highest value. In order not to



Table 13.

pH	maltose		pH	maltose	
	mg	%		mg	%
3.17	0.3	4	5.74	6.4	75
3.25	0.5	6	6.02	6.2	73
3.37	0.8	9	6.24	5.9	69
3.60	1.8	21	6.37	5.6	66
3.78	4.5	53	6.78	4.8	56
4.21	5.7	67	6.80	4.4	52
4.69	8.5	100	7.12	4.0	47
5.17	7.0	82	7.45	3.2	38
5.54	6.7	79	7.77	2.7	32
5.59	6.5	76	8.20	0.4	5

get the quantities of maltose too small, the dextrinogenamylase solution is used at a dilution of 5:50 instead of 2:50. In Fig. 13, the curves represent the relation previously found between concentration of hydrogen ions and the activities of the saccharogenamylase (fully drawn) and dextrinogenamylase (dotted line) respectively, the points representing the values from Table 13.

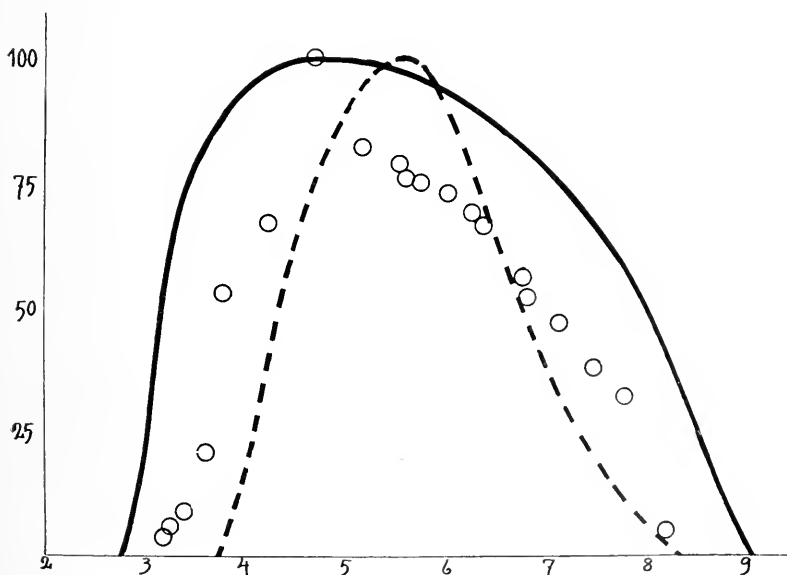


Fig. 13.

Sugar-forming capacity of dextrinogenamylase at different concentrations of hydrogen ions.

The values found lie neither on the curve for saccharogenamylase nor on that for dextrinogenamylase. But it should be borne in mind that the observations in Table 13 were carried out according to the same method as the observations on which the curve for saccharogenamylase is based whereas the observations on which the curve for dextrinogenamylase is based were made according to an entirely different method, which may perhaps explain to some extent the difference found between the observations in Table 13 and the curve for dextrinogenamylase. It may therefore seem warrantable to regard this discrepancy as less important than that relative to the saccharogenamylase curve.

The experiments in Table 13, in conjunction with the data from Exp. VII: 5 etc. thus afford no actual proof, but only suggest it as likely that the formation of reducing substances in these cases is not due solely to a small residual quantity of saccharogenamylase, but either solely or in part to the action of the dextrinogenamylase.

Similar conditions are found to exist in respect of the saccharogenamylase solutions. All of them exhibit a certain activity of dextrinogenamylase; very slight, it is true, but which nevertheless does not disappear completely until the sugar-forming capacity has dropped to very near zero. Take for instance Exp. I: 5. We find here, that X and Y sink rapidly to about one-tenth of their original values, after which, however, the further decrease proceeds very slowly indeed. We find the same phenomenon throughout where the dextrinogenamylase is noticeably inferior in stability to the saccharogenamylase. In certain cases, it is true, as for instance in Exp. I: 4, we have  $X = 0$  after a very short time; this however, does not mean that there is no activity, only that the effect under these conditions is too slight to be measured. The Y values here afford a better idea of the position. The starch hydrolysis does, it is true, proceed as far as the point where the solution is no longer stained blue by iodine, but not so far that no colour appears at all. The above noted phenomena may likewise be explained by assuming that the small X and Y values found are due to the action of the saccharogenamylase, and not to that of the dextrinogenamylase.

In this case also, the influence of concentration of hydrogen ions has been investigated. The capacity of a saccharogenamylase solution to hydrolyse starch at different concentrations of

hydrogen ions, as measured by the iodine staining method, is shown in Table 14. Two values are given for concentration of hydrogen ions. One,  $p_H(X)$  refers to the last colourless tube, the other,  $p_H(Y)$  to the last violet coloured tube.

Table 14.

$p_H(X)$	X	% (X)	$p_H(Y)$	Y	% (Y)
3.14	3	25	3.37	190	100
3.63	12	100	3.68	190	100
4.51	12	100	4.47	190	100
5.99	12	100	6.00	190	100
6.70	12	100	6.74	190	100
7.42	6	50	7.78	100	53

Fig. 14 shows how these X and Y values lie in relation to the curves previously found, indicating the influence of the concentration of hydrogen ions on the activity of the two enzymes.

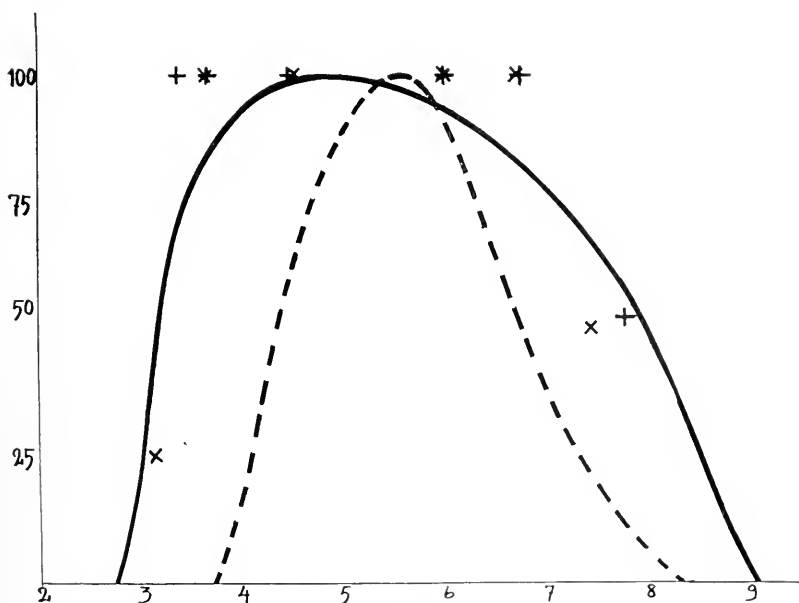


Fig. 14.

Starch-dissolving capacity of saccharogenamylase at different concentrations of hydrogen ions.

x = X.

+ = Y.

The divergence from the curve for saccharogenamylase lies throughout within the limits of experimental error, but there is not the slightest indication of agreement with the curve for dextrinogenamylase. In this case, then, we may consider it proved that the activity is exclusively, or at any rate mainly, due to the effect of the saccharogenamylase. Hence it follows that the saccharogenamylase solutions must be practically pure, i. e. free from dextrinogenamylase.

### 10. The Hydrolysis of Starch.

The present investigations may perhaps also afford some contribution to the study of the hydrolysis of starch<sup>1</sup>). According to one hypothesis, the decomposition of starch is effected by the breaking up of the starch molecules into large fragments, dextrans, which are subsequently again desintegrated into smaller particles that may perhaps still claim to be called dextrin; this process is repeated until the final subdivision produces sugar molecules. According to another hypothesis, the process of hydrolysis takes place thus: maltose molecules are thrown off directly from the starch molecule, which thus divides into a molecule of maltose and a molecule of dextrin. The dextrin is further affected in like manner, and at last disappears altogether. This latter view is perhaps the more generally accepted, owing mainly to the fact that sugar can be shown to occur at a very early stage of the hydrolysis, a fact which better agrees with the latter hypothesis than with the first.

Up to the present, however, all investigations have been carried out with ordinary diastase, i. e. a mixture of two different enzymes. We might therefore expect to find a simpler state of things if the enzymes were taken separately. By investigating the alterations in osmotic pressure which take place during hydrolysis of the starch, I have arrived at the conclusion that the two hypotheses above mentioned are equally correct, the former explaining the action of the dextrinogenamylase, the latter that of the saccharogenamylase.

The investigations were carried out with an osmometer ac-

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<sup>1</sup>) According to Pringsheim (*Die Polysaccharide*. Berlin 1923) and others, the decomposition of starch consists not only in a process of hydrolysis, but also involves depolymerisation.

cording to Sørensen<sup>1</sup>). The membrane was formed of a collodium film, impermeable to soluble starch. The inner vessel contained the solution to be examined, the outer containing the solvent. The solution contained a phosphate buffer. Its salts are diffused through the membrane until the concentration is the same on both sides. The osmotic pressure of the salts is then the same in inner and outer liquids, and thus does not affect the measurement. The measured osmotic pressure then depends solely upon the substances which cannot be diffused through the membrane.

The initial material was a 2 % solution of soluble starch. As however, starch contains water, the true starch content was determined by hydrolysing a portion of the solution to glucose, which was afterwards determined according to Bang. It was found that the solution contained 1.67 % water-free starch. With water as the outer liquid, this starch solution exhibited an osmotic pressure of 58 mm water.

The starch solution was hydrolysed by the action of different enzymes. In Exp. 1, 10 cc of  $m/15$  solution of primary potassium phosphate, and 0.2 cc saccharogenamylase solution were added to 50 cc starch solution. After digesting for 30 minutes at 38°, the solution was heated to boiling point, thus destroying the enzyme. The solution thus obtained was stained a deep blue by iodine. 10 cc had a reduction capacity of 44.9 mg maltose.

Exp. 2 as carried out in a similar manner, but using dextrinogenamylase solution. In order to give the solution approximately the same reduction capacity as in Exp. 1, the hydrolysis was allowed to proceed for abt. 3 hours, after which the liquid was boiled. This solution gave, with iodine, a violet colour. 10 cc had a reduction capacity of 34.4 mg maltose.

These two solutions were examined in the osmometer, using for the outer liquid a solution of potassium phosphate of the same concentration as the inner liquid. Should any slight difference in concentration exist, it is soon eliminated by diffusion. Measurements were not made until after the lapse of 48 hours, in order to allow time for the crystalloids to fall into equilibrium. The temperature was 18°. The results are given in the following table.

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<sup>1</sup>) Studies on Proteins. Comptes-rendus du Lab, Carlsberg **12**, 298 (1917).

Days from commence- ment of experiment	Osmotic pressure in mm	
	Exp. 1	Exp. 2
2	38	407
3	30	—
5	—	282
6	—	257
7	—	227

If no hydrolysis had taken place, the starch solution should, at this dilution, have had an osmotic pressure of 48 mm water.

According to the theory put forward above, the hydrolysis in Exp. 1, under the influence of the saccharogenamylase, should have been effected by a gradual separation of sugar molecules from the starch molecules, the residue consisting of dextrin. Thus one molecule of starch forms one molecule of dextrine. Since the maltose molecules can diffuse through the membrane, they do not affect the osmotic pressure once equilibrium has been established. The number of molecules which cannot diffuse through the membrane should thus not increase, and the osmotic pressure should therefore remain unaltered. Actually, we find a slight decrease, from 48 to 30 mm. This can evidently be due to the fact that some of the starch molecules have become hydrolysed to such an extent that all the products formed are capable of diffusion.

In Exp. 2, the starch should, by the action of the dextrinogenamylase, be decomposed into a number of relatively large molecules, and the osmotic pressure should therefore be increased. We also find a considerable increase, from 48 to 407 mm, after which however, the osmotic pressure again decreases. This is very likely due to the fact that the osmotically active elements are in reality capable of permeating through the membrane, albeit very slowly.

The composition of the liquids on either side of the membrane also agrees with what we should expect from our assumption. In Exp. 1, the reduction capacity should depend entirely on the occurrence of maltose, and we cannot expect to find any considerable quantity of low molecular or reducing dextrans. The reduction capacity in the liquids on either side of the membrane should therefore be approximately equal, since the maltose is able to diffuse without hindrance. The reduction capacity expressed in mg maltose per 100 cc liquid was, for the inner liquid,

28, and for the outer, 20. In both liquids, the carbohydrate was subsequently transformed into glucose by boiling with hydrochloric acid. This gave, in the outer liquid, 22 mg glucose per 100 cc. Prior to the hydrolysis with hydrochloric acid, the outer liquid contained 20 mg maltose, from which 21 mg glucose was formed. As the determination gives 22 mg, the discrepancy is only 1 mg which lies within the limits of experimental error. We find then, in the outer liquid, only maltose, but no other carbohydrate. After hydrolysis of the inner liquid on the other hand, we find 1300 mg glucose per 100 cc, which is an unprecedented increase of the reduction capacity. Of this great quantity of glucose, only 22 mg can have arisen through hydrolysis of maltose, seeing that the concentration of maltose should be the same on both sides of the membrane. The remainder must have been produced by hydrolysis of high-molecular, non-reducing or slightly reducing carbohydrates.

In Exp. 2, we find, in the inner liquid, a reduction capacity of 74 mg maltose per 100 g, but in the outer liquid, a reduction capacity of only 18 mg. The high reduction capacity of the inner liquid can only in part be due to the presence of maltose, since this passes through the membrane, and should thus be of the same concentration on both sides. The reduction capacity must be partly accounted for by the presence of carbohydrates, which cannot diffuse through the membrane, i. e. reducing dextrans.

On hydrolysing the outer liquid with hydrochloric acid, we find 62 mg glucose per 100 cc. The amount corresponding to 18 mg of maltose — the reduction capacity prior to hydrolysis, — is only 19 mg glucose. There must then, have been other carbohydrates in the outer liquid besides the maltose; indeed, it is possible that it contains only reducing dextrans, and no maltose at all.

In the inner liquid, we find, after hydrolysis, with hydrochloric acid, 760 mg glucose as against 74 mg maltose before hydrolysis. The increase in reduction capacity is thus less than in Exp. 1, which is a natural consequence of the fact that a greater quantity of carbohydrates has diffused out through the membrane.

That the hydrolysis proceeds in different ways through the action of saccharogenamylase or dextrinogenamylase is also evident from investigation of the starch-dissolving capacity of the

enzyme. In all cases where the hydrolysis undoubtedly depends on the presence of dextrinogenamylase, we find a fairly constant proportion between the values X and Y, the latter being about 6 to 8 times to value of X. On the other hand, we find a greater difference between X and Y in all cases where there is reason to attribute the alteration in iodine colouring to the effect of saccharogenamylase. Y can in this case amount to as much as 64 times the value of X. Cf. the experiments in Chap. 5.

From the point of view of the hypothesis above noted, we can interpret this as follows. When, through the action of saccharogenamylase, a certain number of sugar molecules have been formed, there is still a certain residue, dextrin, which is stained violet by iodine. Only when this reaction has proceeded still further, and to a relatively considerable extent, i. e. only when a relatively large number of sugar molecules are formed from one starch molecule, does the residual dextrin lose the power of being coloured by iodine. If, on the other hand, the hydrolysis be effected by dextrinogenamylase, then the starch molecule is broken up into two or more equal parts, and a repetition of this process soon brings us to products no longer stained by iodine. In these two cases, then, the dextrans are formed in altogether different ways, and they can therefore hardly be identical. I fancy also, that there is a slight difference in the colour produced by the iodine. When we investigate, in the usual manner, the activity of a solution of dextrinogenamylase, we obtain a number of tubes which turn a brownish violet under the application of iodine. The same shades of colour are obtained with unaltered malt solution, whereas when saccharogenamylase is treated in this manner, the colours produced are more of a pure violet. The difference, however, is not great, and if more iodine be added, the colour will in this case also tend more towards the brown.

## **11. Hydrolysis of ordinary starch and of soluble starch.**

### **Combined action of enzymes.**

All the experiments above described were made with soluble starch. I give below a description of some comparative experiments made partly with soluble starch, partly with ordinary potato starch of the same concentration.

In investigating the sugar formation, 4 distinct experiments were made with each kind of starch, using different enzymes, viz:



1. Saccharogenamylase diluted to 2 : 50.
2. Dextrinogenamylase diluted to 2 : 50.
3. A mixture of 2 cc each of these enzymes to 50 cc.
4. The original malt solution from which these enzymes were prepared, diluted 1 : 100. As the malt solution, in the preparation of the two enzymes, is diluted in the proportion of 1 : 4, the dilution in this case will also be the same as in 1 and 2. The results will be seen from Table 15. The figures denote mg maltose.  $p_{11}$  was in all experiments 6.1.

Table 15.

	Soluble starch	Ordinary starch
1. Saccharogenamylase .....	27.5	18.1
2. Dextrinogenamylase .....	4.3	1.4
3. Saccharogenamylase + dextrinogenamylase ....	31.3	24.0
4. Original malt solution .....	36.9	27.8

In all the experiments, considerably less maltose was formed by the action of the enzymes with ordinary starch than with soluble starch. It is further apparent from the experiments that we cannot, by mixing the solutions of saccharogenamylase and dextrinogenamylase, get back the whole activity possessed by the original malt solution. In the preparation of saccharogenamylase we lose, not only the dextrinogenamylase, but also a small amount of the saccharogenamylase itself.

These experiments may also afford an answer to the question as to how far combined action exists between the enzymes or not. It has hitherto been generally supposed that if the malt diastase consists of two different enzymes, then the one should hydrolyse the starch to dextrin, and the other hydrolyse the dextrins to maltose; or in other words, that the enzymes should be an amylase and a dextrinase (cf. Chap. 1). If this were correct, then the quantity of sugar formed by the simultaneous action of both enzymes on a starch solution should be essentially greater than the sum of the quantities formed by the enzymes individually under the same conditions. In the experiments with soluble starch, Table 15, the enzymes individually formed  $27.5 + 4.3 = 31.8$  mg maltose, but by their combined action 31.3.

The difference lies within the limits of experimental error. Using ordinary starch, the enzymes formed, individually  $18.1 + 1.4 = 19.5$  mg maltose, jointly, 24.0 mg. In this case, then, we have a considerably greater quantity of maltose formed by joint action of the two enzymes than when each is acting on its own account. But allowance has yet to be made for an important factor, to wit, the viscosity of the solutions. In a solution of ordinary starch, the viscosity is greater than in a solution of soluble starch, but through the action of the dextrinogenamylase, the viscosity soon falls to about the same value in both solutions, and for this reason alone, the dextrinogenamylase should assist the saccharogenamylase in sugar-forming capacity to a greater extent in solutions of ordinary starch than in solutions of soluble starch. Even disregarding the effect of alteration in viscosity, however, the fact remains that saccharogenamylase, under the given experimental conditions, is able to form by itself at least two-thirds of the amount of sugar formed by simultaneous action of both enzymes. The saccharogenamylase cannot therefore be said to be a dextrinase, for it attacks unaltered starch as well as dextrin. That the reaction velocity is higher in the hydrolysis of dextrans than in the hydrolysis of starch, may be due partly to the difference in viscosity of the substrate solutions, but partly also to the fact that a dextrin solution contains a greater number of molecules than a starch solution of like concentration.

That soluble starch by the action of dextrinogenamylase is also rendered more easily affected by saccharogenamylase is shown by the following experiment. To 100 cc 2 % soluble starch was added 40 cc phosphate »1 secondary« and the whole heated to  $65^{\circ}$ . 1 cc dextrinogenamylase was added. After 15 minutes, the solution was heated to boiling point and the enzyme thereby destroyed. The dextrin solution thus obtained gives with iodine a light claret colour. To 35 cc of this dextrin solution is added 5 cc saccharogenamylase diluted to 2:50. The quantity of sugar formed is determined in the usual manner after 30 minutes digestion at  $38^{\circ}$ . At the same time, a similar experiment was carried out but without dextrinogenamylase. In addition, two control experiments were carried out with boiled saccharogenamylase.

The following values were obtained:

	Maltose	Control exp.	Newly formed maltose
Dextrin solution . . .	54.7	25.3	29.4
Starch solution . . . .	29.8	4.7	25.1

The difference is distinct, though slight when we consider the extensive hydrolysis which has already taken place in the preparation of the dextrin solution.

It is thus apparent from these experiments, that the saccharogenamylase is capable of attacking ordinary starch, soluble starch and dextrin. The quantity of maltose formed is least in the first case and greatest in the last, but the difference is very slight, and might perhaps at least to some extent be explained by the difference in viscosity of the solutions, and the difference in sizes of the molecules of the substrates.

Similar experiments have also been carried out with the iodine staining method. The concentration of hydrogen ions was  $p_H = 6.0$ . The experiments were carried out according to Series A (cf. Chap. 2).

	Soluble starch		Ordinary starch	
	X	Y	X	Y
Original malt solution . . . . .	190	1500	0	770
Dextrinogenamylase . . . . .	100	770	0	380
Saccharogenamylase . . . . .	12	190	0	100

Using ordinary starch, the Y values become about half those obtained with the soluble starch, and the transition in shade from blue to bluish violet it about the same in both cases. Ordinary starch on the other hand, gives X values considerably below those obtained with soluble starch. Actually, in the experiments with ordinary starch, we obtain no colourless tubes, but by using larger quantities of enzyme than those answering to Series A, we could here also, with the ordinary starch, obtain a pale yellow colour with the iodine. The shade of colour in the violet tubes likewise differs from that obtained when using soluble starch. The last violet tube was of a violet strongly tinged with blue, and the same colour was also found in the preceding tubes with almost the same quality, only still paler. The line of

demarcation between the last colourless and the first violet tube, which is sharply defined with soluble starch, is here replaced by an almost continuous transition.

It is noticeable that the first stage of the hydrolysis, answering to the iodine alteration from pure blue to bluish violet, proceeds but very little slower in the case of ordinary starch than with soluble starch, the continued hydrolysis of the ordinary starch being greatly retarded. In any case, however, the proportion between the effects of the two enzymes is the same.

*April 1926.*

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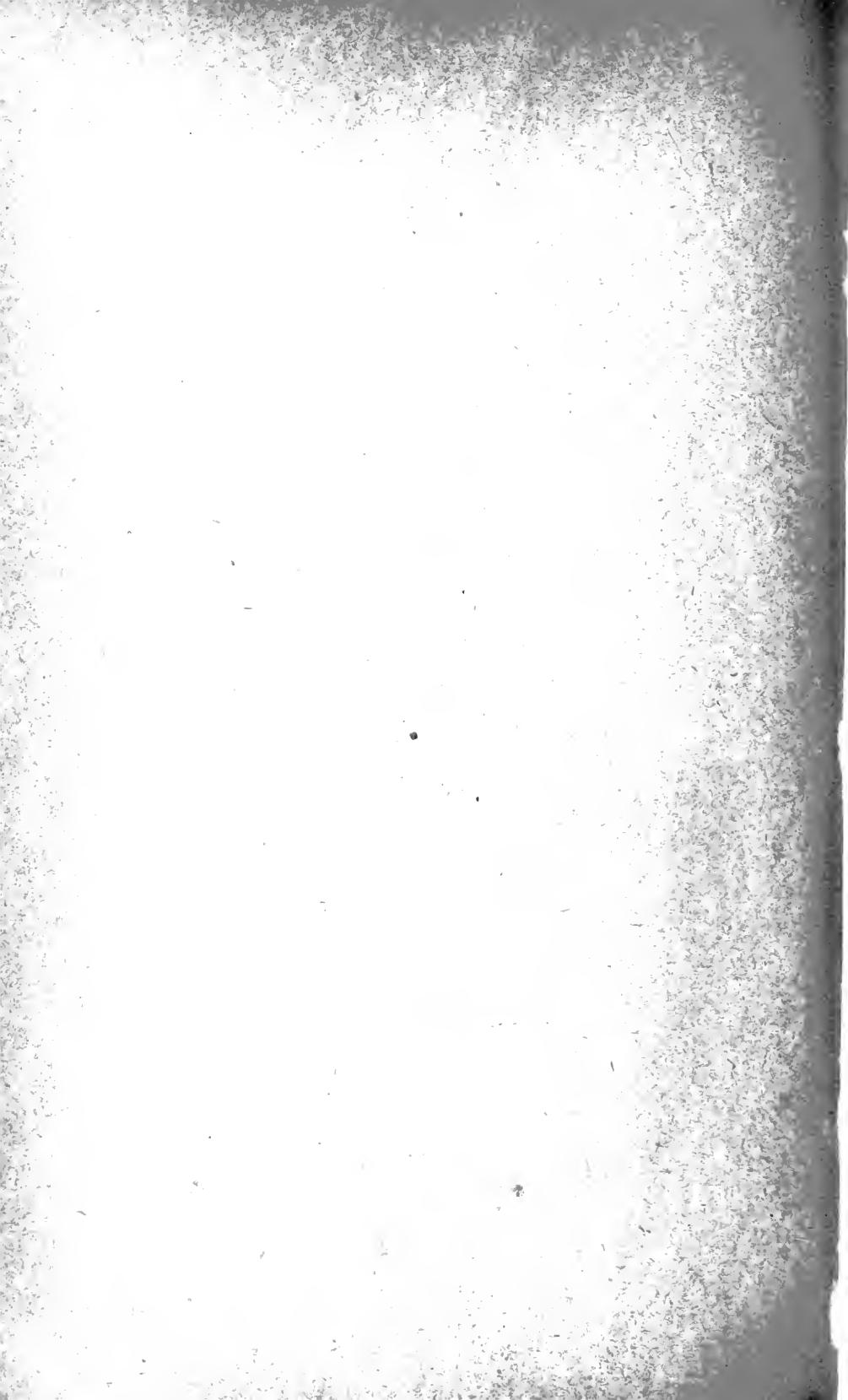
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# SUR LA COMPOSITION ET LA CARACTÉRISATION DES PROTÉINES NATURELLES<sup>1)</sup>.

PAR

S. P. L. SORESENSEN.

Si j'ai choisi comme sujet de cette conférence la question de la composition et la caractérisation des substances protéiques naturelles, ce n'est pas qu'il soit maintenant possible d'y donner une réponse satisfaisante. Cela demande encore beaucoup de temps et de travail; cependant, c'est vraiment un problème palpitant d'intérêt et d'actualité, et la discussion de la manière dont ces substances si intéressantes et si importantes paraissent être constituées est en ce moment si animée et présente des aspects si variés, que j'ai pensé qu'un court aperçu des divers points de vue auxquels les différents chercheurs se sont placés pourrait être de quelque intérêt pour une réunion de chimistes scandinaves.

La question de la composition des substances protéiques est d'une nature double. En premier lieu, on peut se demander quels sont les éléments qui entrent dans la composition des protéines, et dans quelles proportions; c'est ce qu'on appelle la composition centésimale «élémentaire». Une fois cette question résolue, la seconde sera tout naturellement celle-ci: Comment sont liés ensemble les différents atomes d'élément dont se compose la molécule de protéine ou le complexe de molécules protéiques? en d'autres termes: quels sont les composés de structure plus simple dont l'ensemble constitue les substances protéiques? Considérons d'abord ce dernier côté du problème.

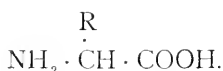
Pendant la première moitié du dernier siècle, la théorie formulée par le Hollandais Mulder était universellement reçue.

<sup>1)</sup> Conférence faite au troisième Congrès des Chimistes Scandinaves, à Helsingfors (séance d'ouverture, le 13 juillet 1926).

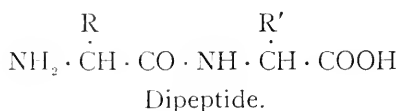
D'après elle, toutes les albumines seraient formées d'une seule et même matière organique azotée, à laquelle des «actions vitales» auraient fait subir des modifications différentes et qui, en outre, se serait associée avec des substances inorganiques en proportions variées. Supposant que cette matière azotée commune à toutes les albumines formait la base du maintien des actions vitales, Mulder donna à cette «*prima materia*» le nom de «protéine», dérivé du verbe grec qui signifie occuper la première place.

A mesure qu'augmentèrent nos connaissances sur les différentes propriétés des diverses substances protéiques, la théorie de Mulder sur l'existence d'une matière première, fondamentale, commune à toutes les protéines, fut délaissée, et l'on commença de porter l'attention sur les produits de scission qu'on peut obtenir en décomposant les protéines soit à l'aide d'un traitement par des acides ou des alcalis, soit au moyen des dédoublements enzymatiques.

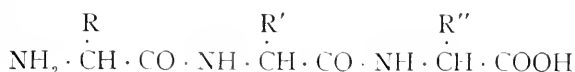
Dès 1820 environ, Proust et Braconnot, parmi les produits dérivés des muscles et de la colle au moyen de scission par des acides, avaient isolé respectivement la leucine et la glycolle, et peu avant 1850 Liebig y ajouta la tyrosine, obtenue en décomposant la corne par des acides. C'est seulement en 1865 que Cramer parvint à isoler un quatrième produit de dédoublement: la sérine, tirée de la colle de soie. Dans la suite, on trouva — souvent à plusieurs années d'intervalle — encore d'autres produits de scission, et à l'heure actuelle on connaît une vingtaine de produits différents obtenus par décomposition de protéines. Ces produits sont en partie extrêmement dissemblables les uns aux autres, mais, sauf une seule exception, ils ont ceci de commun, qu'ils sont des acides aminés, de sorte qu'ils peuvent être représentés par cette formule:



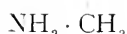
Ainsi que Emil Fischer l'a démontré systématiquement au début du siècle courant, ces acides aminés peuvent, moyennant des réactions chimiques appropriées, s'unir avec élimination d'eau et formation des combinaisons nommées peptides:







Tripeptide.



Reste de lysine.

Reste de glutamine.

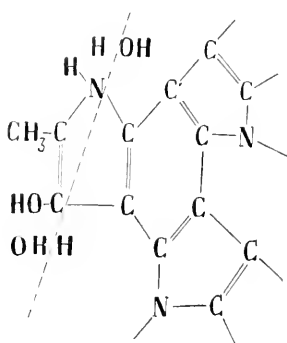
Tripeptide.

Or, comme ces peptides préparées par synthèse, et en particulier ceux de structure compliquée, ressemblent aux produits relativement peu dégradés des protéines, produits communément appelés peptones, il semble légitime d'admettre que les protéines consistent essentiellement en des acides aminés réunis entre eux par des liaisons de peptides: — CO · NH —.

Cette conception de la structure des substances protéiques — conception qui s'appuie surtout sur les recherches étendues et minutieuses d'Emil Fischer — donne une explication simple et naturelle de plusieurs propriétés importantes des protéines, et tout particulièrement des nombreuses analogies constatées entre celles-ci et les acides aminés, ainsi que du fait que ces derniers naissent de la scission des protéines. Aussi, depuis de nombreuses années, la manière de voir de Fischer constitue-t-elle le fondement de toutes les considérations touchant la composition et la structure des protéines, et ce ne sont que les investigations poursuivies pendant ces années dernières qui semblent nécessiter une revision de cette théorie.

A ce propos, je citerai le chimiste danois N. Troensegaard qui, il y a cinq ou six ans, avançait la manière de voir que les substances protéiques sont constituées par des anneaux hétérocycliques hydroxylés, et principalement des anneaux pyrroliques. Troensegaard pense ainsi faire le pont conduisant des protéines, d'un côté, jusqu'aux composés à pyrrol, de l'autre, c'est-à-dire l'hémine de sang et la chlorophylle des plantes. Pour re-

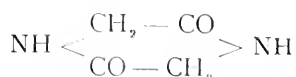
présenter le modèle provisoire d'un système d'anneaux protéique, Troensegaard propose la formule que voici :



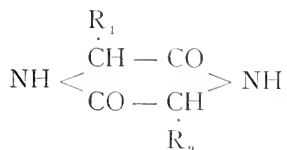
Quant à la formation d'acides aminés par hydrolyse, Troensegaard se représente qu'elle a lieu de la manière indiquée par la ligne pointillée de la figure. On voit qu'une pareille hydrolyse — qui exige la complète rupture de l'anneau de pyrrol — conduira à la formation d'alanine ou, en cas de substitutions ayant eu lieu dans le groupe méthylque, à des alanines substituées, telles que phénylalanine, tyrosine, tryptophane ou histidine. Depuis, au moyen d'une série d'expériences mûrement réfléchies et soigneusement exécutées, plus particulièrement des expériences diverses de dédoublement après réduction, Troensegaard s'est efforcé de prouver la justesse de ses idées; selon moi, cependant, il n'y a pas réussi d'une façon décisive. Il est pourtant hors de doute — et déjà Emil Fischer s'en rendait parfaitement compte — que dans la molécule de protéine il existe d'autres liaisons que celles de peptides seules, et il est plus que probable qu'une partie de l'azote qu'une décomposition hydrolytique ordinaire n'a pas encore permis d'isoler sous forme de composés connus, existe dans des anneaux hétérocycliques facilement décomposables, pareils ou semblables à ceux dont parle Troensegaard. Néanmoins, c'est sans doute aller trop loin que de vouloir remplacer toutes les liaisons peptidiques par des anneaux hétérocycliques de cette espèce.

Une hypothèse qui se rapprocherait davantage de la conception habituelle selon laquelle les substances protéiques sont regardées comme des polypeptides compliqués, serait celle qui admettrait la présence d'anneaux de dicétopipérazine dans la

molécule de protéine. La plus simple de ces combinaisons est, on le sait, l'anhydride de glycolle:



tandis que la formule ordinaire d'un anhydride de deux différents acides aminés peut s'écrire:



La possibilité de la présence de pareils groupes dans la molécule protéique, n'était point étrangère à Emil Fischer, et Emil Abderhalden a également examiné cette question à plusieurs reprises. Elle n'est cependant devenue d'actualité que depuis le moment où — peu après 1920 — s'appuyant sur des recherches spectrographiques d'après Röntgen portant sur des substances organiques à grosses molécules et particulièrement sur la fibroïne de soie, R. O. Herzog et ses collaborateurs ont cru pouvoir démontrer que la substance fondamentale de la partie essentielle de la fibroïne est un polypeptide de structure simple ou bien un anhydride de celui-ci, ne contenant que du glycolle et de l'alanine. D'après ces chimistes, ce composé serait vraisemblablement un anhydride de glycylalanine, donc un anneau de dicétopipérazine. Quant aux molécules de la substance fondamentale, Herzog estime qu'elles seraient liées entre elles par des valences secondaires.

Depuis lors, la question relative à la présence d'anneaux de dicétopipérazine et d'autres anneaux semblables dans la molécule de protéine a fait l'objet d'une vive discussion et de recherches expérimentales approfondies, surtout de la part d'Abderhalden, de M. Bergmann et de P. Karrer. Cela nous mènerait trop loin d'entrer dans tous les détails de ce problème délicat et ardu, qui est encore à l'heure actuelle loin d'être complètement tranché. Je voudrais seulement mentionner quelques points intéressants.

C'est tout particulièrement Abderhalden qui a fait des efforts persévérants pour démontrer la présence de dicétopipérazines dans les protéines. A cet effet, il a non seulement tâché d'isoler — et il y est parvenu — de tels corps parmi les produits

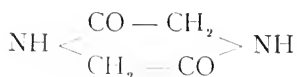
de décomposition des protéines, mais il a aussi cherché à prouver que ces anhydrides ne sont pas formés de polypeptides par un processus secondaire. Abderhalden a encore étudié un certain nombre de réactions colorées comme aussi des processus d'oxydation et de réduction, où les polypeptides et les dicétopipérazines se comportent différemment. C'est ainsi que par des réductions appropriées les dicétopipérazines donnent des pipérazines, et il en est de même pour les protéines qu'il a étudiées; mais les polypeptides ne le font pas, et il en tire la conclusion que les protéines doivent renfermer l'anneau de dicétopipérazine.

Les expériences d'Abderhalden et son interprétation de leurs résultats ont fait l'objet de quelques critiques, non sans raison, ce me semble. Il m'apparaît qu'on peut dire tout au plus que ses travaux appuient la théorie d'après laquelle tout au moins une partie de l'azote protéique aurait pris la forme d'anneaux de dicétopipérazine.

Parmi les diverses questions qu'a soulevées la discussion de la structure des substances protéiques, l'une des plus importantes est celle relative à la manière dont les composés dont il s'agit se comportent vis-à-vis des enzymes protéolytiques. Waldschmidt-Leitz et Schäffner<sup>1)</sup> ont maintenant montré qu'aucun des enzymes protéolytiques ordinaires ne s'attaque aux dicétopipérazines, alors que, comme on le sait, les polypeptides sont facilement décomposés par l'érepsine. Cette observation porte évidemment à admettre qu'aucune partie essentielle de la molécule protéique ne peut être formée de dicétopipérazines, du moins pas dans leur forme habituelle, la forme cétonique. Les dicétopipérazines sont, en somme, des substances trop peu actives, trop peu labiles pour pouvoir sous cette forme former la partie principale de substances aussi sujettes à varier que le sont les protéines. Aussi, Abderhalden a-t-il fait observer cette possibilité, que les dicétopipérazines n'existent pas sous la forme ordinaire, cétonique, mais bien dans une forme tautomère, plus labile. Effectivement, en les chauffant fortement en présence d'aniline, il a pu transformer des dicétopipérazines ordinaires en des composés tautomères plus actifs et présentant des propriétés qui dénotent la présence d'une liaison double. Abderhalden donne les formules de ces dioxopipérazines 2.5 « desmotropes » :

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<sup>1</sup> Ber. 58, 1356 1925.

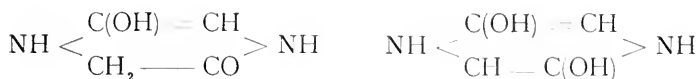


Forme cétonique.



Dihydro-dioxy-Pyrazine

Forme énolique.



Formes nouvelles.

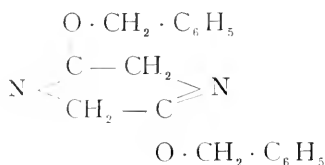
Abderhalden pense que la forme desmotrope qu'il a préparée présente la double liaison entre les atomes de carbone. Toutefois, il n'a pas de raison décisive pour préférer cette formule à la forme énolique ordinairement admise. Pour ce qui regarde les protéines très résistantes du genre de la fibroïne de soie, des kératines etc., il estime<sup>1)</sup> qu'elles renferment la cétoforme stable, alors que les protéines plus labiles contiendraient l'une des formes d'anhydride à liaison double. Il fait ressortir comme une particularité caractéristique que l'anhydride de glycine, corps très labile préparé par lui, se trouve converti en la forme cétonique déjà par chauffage en dissolution aqueuse vers 90—100 degrés centigrades, et il est incontestable qu'une telle réaction rappelle la dénaturation des protéines par chauffage.

Se plaçant à des points de vue semblables et se basant sur les résultats obtenus par des recherches röntgenspectrographiques sur des substances à grosses molécules, plusieurs autres savants ont également étudié la question des anneaux azotés labiles constituant la substance fondamentale des protéines. Je vais résumer quelques-unes des plus importantes de ces recherches

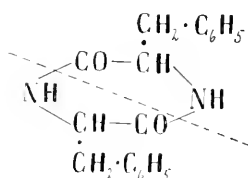
Karrer, Gränacher et Schlosser<sup>2)</sup> ont montré qu'en traitant l'anhydride de glycine argenté par le chlorure de benzyle au bain d'eau bouillante, on arrive à la formation de l'éther OO'-dibenzyle de la dioxidihydro-pyrazine :

<sup>1)</sup> Zeitschr. physiol. Chem., **152**, 89 (1925).

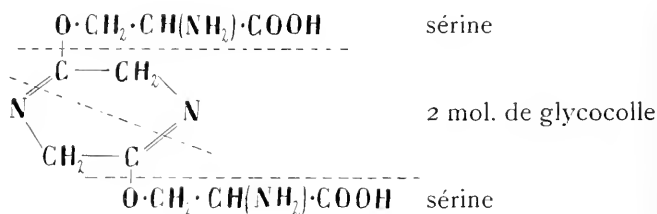
<sup>2)</sup> Helvetica chimica acta, **6**, 1108 (1923).



lequel offre des propriétés toutes différentes de celles qui caractérisent l'anhydride bien connu de la phénylalanine:

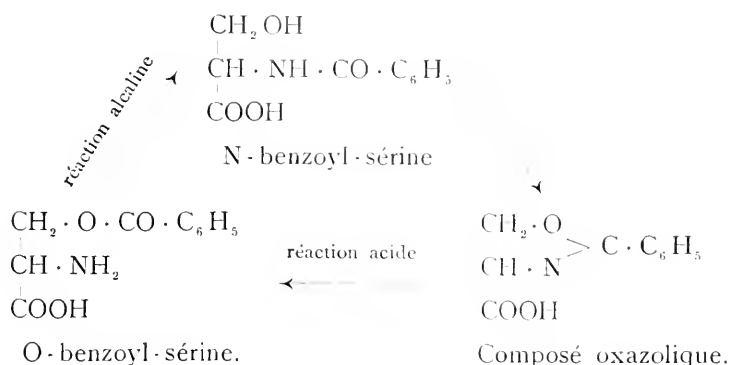


Au contraire de l'anhydride de phénylalanine, la combinaison nouvelle se décompose très facilement par chauffage dans un liquide acide, avec formation de glycolle et d'alcool benzylique. Les auteurs susnommés appellent l'attention sur la grande labilité et force de réaction dont sont doués de tels composés en comparaison des anhydrides ordinaires des acides aminés, et ils ajoutent: «Die Vorstellung, dass ein physiologisch so ungemein aktiver und wandelbarer und chemisch labiler Stoff wie das Eiweiss nur aus verhältnismässig reaktionsträgen und beständigen Polypeptidketten und Diketopiperazinringen aufgebaut sei, kann schwerlich befriedigen.» C'est surtout les acides oxyaminés qui prennent naissance par une simple hydrolyse des composés du type indiqué par ces auteurs, ainsi qu'il appert de la formule ci-après, représentant une substance qui par hydrolyse donnera de la sérine et du glycolle:



Sous ce rapport, une série d'importants travaux expérimentaux exécutés par M. Bergmann et ses collaborateurs présente un grand intérêt. Comme un exemple très clair et net, je citerai

les recherches effectuées par Bergmann et Mickeley<sup>1)</sup> sur les conditions de transformation de la benzolsérine, lesquelles peuvent s'exprimer par le schéma suivant:



Si la N-benzoyl-sérine ordinaire est traitée par la diazométhane, on verra l'éther méthylique prendre naissance, et l'on peut alors traiter ce composé par le chlorure de thionyle, lequel déjà à froid fournira avec séparation d'eau, une combinaison d'oxazoline stable en milieu alcalin, et qui par conséquent peut être saponifiée avec formation d'un sel du composé d'oxazoline indiqué dans le schéma. Cependant, dès que le liquide sera rendu acide, il y aura un commencement d'hydratation avec formation d'O-benzoyl-sérine. Cette combinaison n'est stable qu'en milieu acide; aussitôt que celui-ci deviendra alcalin, elle se convertira par transposition en la N-benzoyl-sérine primitive. Nous n'avons pas à discuter ici la cause de ces transpositions; je me bornerai à mentionner que, si la benzoyle de la N-benzoyl-sérine est remplacée par le radical du glycocolle  $\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CO} -$ , on arrive à la formule de la glycyl-sérine ordinaire, corps que Bergmann a également étudié. Les conditions sont ici plus compliquées; néanmoins, ici encore on est parvenu à faire naître des combinaisons qui au moyen d'une simple modification de la réaction de la solution se convertissent en substances isomères. Bergmann dit à ce sujet: «Ich halte es für sehr wahrscheinlich, dass solche Oxazoline oder analoge instabile Ringsysteme am Aufbau der natürlichen Proteine beteiligt sind.»<sup>2)</sup>

<sup>1)</sup> Zeitschr. physiol. Chem., **140**, 128 (1924).

<sup>2)</sup> Die Naturwissenschaften, **12**, 1158 (1924).

Les travaux très récents que je viens de résumer d'une façon très succincte et très incomplète, présentent certainement un grand intérêt. C'est à bon droit que leurs auteurs font ressortir combien il est souhaitable, nécessaire même, pour l'intelligence de nombreuses transpositions subies par les substances protéiques, de pouvoir constater l'existence de liaisons labiles dans la structure de ces corps: il n'est besoin de citer que le processus de dénaturation, réaction encore aujourd'hui obscure que subissent un grand nombre de protéines solubles, par ex. à la suite d'un chauffage ou d'un traitement à l'alcool.

Il se pose cependant une question qu'à mon avis il est indispensable de soumettre à un examen plus approfondi avant de pouvoir tirer des conclusions définitives et décisives au sujet des idées nouvelles sur la structure des protéines: c'est la question de savoir comment des composés qu'on suppose avoir telle ou telle structure se comporteront vis-à-vis des enzymes protéolytiques. A ma connaissance, tout ce qu'on en sait, c'est, comme je l'ai déjà dit, que les dicétopipérazines, au contraire des polypeptides, ne sont pas décomposées par l'érepsine. La question peut maintenant se poser avec d'autant plus de précision, que les investigations effectuées pendant ces années dernières par Waldschmidt-Leitz et ces collaborateurs dans le laboratoire de Willstätter à Munich, ont contribué puissamment à éclaircir les effets spécifiques des enzymes protéolytiques. Au moyen d'adsorption convenablement effectuée des enzymes (l'adsorbant étant de l'oxyde d'aluminium préparé par un procédé approprié), puis élution convenable, on est parvenu à isoler les différents enzymes protéolytiques à un état de plus grande pureté qu'auparavant. Comme le montre le tableau ci-dessous, on a pu ainsi constater que tous les peptides étudiés sont décomposés par l'érepsine, alors qu'aucun d'eux n'est scindé par la trypsine, ni même un tripeptide qui avait été antérieurement regardé comme décomposable par ce dernier enzyme. De plus, on voit que le champ d'activité de l'érepsine ne s'étend qu'aux peptides simples; ni la protamine, ni l'histone ni même aucune des protéines de structure plus compliquée ne se laissent décomposer par l'érepsine. Par contre, toutes ces substances sont scindées par la trypsine activée à l'aide de l'entérokinase; dans aucun cas l'érepsine ne peut remplacer la trypsine, ni celle-ci remplacer celle-la.<sup>1)</sup>

<sup>1)</sup> Ajouté à la correction de la preuve: Toutefois, dans un travail publié tout



## Spécificité des Enzymes.

— = aucune scission. + = sc. ++ = scission augmentée.

N°	Substratum	Enzyme		
		Érepsine	Trypsine	Trypsine + Entérokinase
1	Alanyl-glycine .....	+	—	—
2	Glycyl-tyrosine .....	+	—	—
3	Glycyl-glycine .....	+	—	—
4	Glycyl-alanine .....	+	—	—
5	Leucyl-glycine .....	+	—	—
6	Leucyl-alanine .....	+	—	—
7	Leucyl-glycyl-glycine .....	+	—	—
8	Peptone (ex albumine, Merck) .	—	+	++
9	Clupéine .....	—	+	++
10	Thymushistone .....	—	+	++
11	Caséine .....	—	—	+
12	Fibrine .....	—	—	+
13	Gélatine .....	—	—	+
14	Gliadine .....	—	—	+
15	Zéine .....	—	—	+

Les deux scissions enzymatiques se ressemblent pourtant sur un point: toutes les deux donnent naissance à des groupes carboxylés (mesurables par la titration au formol ou la titration d'après Willstätter) et à des groupes aminés (mesurables par le procédé de van-Slyke ou celui de Folin), et pour les polypeptides simples où un contrôle précis est possible, la formation des groupes carboxylés et aminés se fait en quantités équivalentes. Ceci s'explique — et a été expliqué jusqu'ici — en admettant un dédoublement de liaisons peptidiques; et il n'a point encore

récemment (E. Waldschmidt-Leitz, A. Schöffner et W. Grassmann: Zeitschr. physiol. Chem., **156**, 68 (1926)) cette manière de voir se trouve quelque peu modifiée, en ce sens que, en étudiant les effets produits successivement sur la clupéine par divers enzymes protéolytiques, on constate qu'en variant l'ordre dans lequel ces enzymes sont employés, on arrive à des résultats incompatibles avec l'hypothèse d'une spécificité absolue de chaque enzyme. Il semblerait que l'action spécifique de ceux-ci soit déterminée non seulement par les acides aminés particuliers qui font partie du complexe de polypeptides sur lequel l'enzyme agit, mais aussi par d'autres facteurs inconnus jusqu'ici.

été prouvé que les composés annulaires qui sont supposés faire partie des substances protéiques soient susceptibles d'être scindés par la trypsine ou l'érepsine avec formation de groupes carboxylés ou aminés.

En ce qui concerne la décomposition par la pepsine, il est connu de longue date que l'effet produit est bien moins radical. La plupart des protéines naturelles sont attaquées — du moins, après dénaturation — par la pepsine; mais la décomposition, mesurée p. ex. au moyen de la titration au formol, est très peu considérable, alors que d'un autre côté les propriétés de la substance éprouvent des modifications profondes. Jusqu'ici, on ne sait pas encore avec certitude comment les choses se passent, mais en tous cas l'action ne semble pas se borner à la scission d'un petit nombre de liaisons peptidiques. A cet égard, quelques essais publiés cette année par H. Steudel et ses collaborateurs<sup>1)</sup> présentent un certain intérêt. Ces chercheurs ont trouvé dans la décomposition par la pepsine d'un assez grand nombre de substances protéiques une beaucoup plus forte augmentation des groupes carboxylés que des groupes aminés; mais ils ne peuvent pas donner, ni eux non plus, une explication bien précise de ce fait<sup>2)</sup>.

Dans l'état actuel des choses, il me semble donc prématuré d'admettre que la molécule de protéine se compose principalement d'anneaux hétérocycliques. Il est même vraisemblable qu'à côté des anneaux hétérocycliques bien connus (l'anneau d'indol dans le tryptophane, l'anneau d'imidazol dans l'histidine, et l'anneau de proline), il se trouve de plus faibles quantités d'autres anneaux hétérocycliques; pourtant, je considère toujours la liaison peptidique comme la principale.

Il reste à savoir s'il est raisonnable de se figurer la molécule protéique comme formée par un vaste complexe de polypeptides. Des recherches spectrographiques mentionnées plus haut sur la fibroïne de soie, il paraît résulter que la substance fondamentale de la partie cristalline de ce corps a une structure simple; cependant, il peut être question ici tout aussi bien d'un dipeptide que d'un

<sup>1)</sup> Zeitschr. physiol. Chem., **154**, 21 og 198 (1926).

<sup>2)</sup> Ajouté à la correction de la preuve: Des essais que viennent de publier E. Waldschmidt-Leitz et E. Simons (Zeitschr. physiol. Chem., **156**, 114 (1926)), font toutefois voir que dans la scission par la pepsine de certaines protéines (caséine, albumine d'œuf, globuline de ricin), il se forme — aux premiers stades de la décomposition, au moins — des groupes carboxylés et aminés en quantités équivalentes.

noyau de dicétopipérazine. Il se peut qu'il en soit essentiellement de même — bien que souvent d'une façon notablement plus compliquée — pour d'autres protéines, qui seraient ainsi constituées principalement par des complexes plus ou moins grands de polypeptides, liés ensemble de façon secondaire ou bien par formation réciproque de sels, mais sans qu'une réaction chimique proprement dite eût eu lieu entre les complexes mêmes. De pareils complexes liés ensemble bien que d'une façon peu cohérente, agiraient en dissolution comme un ensemble et montreraient par conséquent une pression osmotique extrêmement faible, telle qu'on la constate en effet. Cette manière de voir fournit aussi l'explication des remarquables propriétés que les albumines et les globulines possèdent en ce qui concerne la solubilité et le fractionnement en solutions salines.

Au cours de notre précédente réunion à Gothenbourg<sup>1)</sup>, j'ai donné un aperçu des solubilités des sérumglobulines, et je croyais pouvoir démontrer que ces phénomènes assez singuliers ne peuvent pas s'expliquer par l'hypothèse d'après laquelle nous avons à faire à des mélanges de globulines différentes, mais uniquement par cette autre hypothèse, que ces divers complexes de globulines sont liés ensemble d'une façon peu cohérente, de manière à former un ensemble auquel j'attribuais la formule  $Ep Pq$ , formule qui devait exprimer brièvement que les différentes globulines renferment un nombre différent de complexes d'euglobulines (E) et de pseudoglobulines (P). Ainsi tous les faits remarquables que présentent les conditions de solubilité et de précipitation se trouveraient expliqués.

Depuis lors Linderström-Lang, au Laboratoire Carlsberg, a, avec succès, adopté la même manière de voir dans une série d'essais de fractionnement portant sur la caséine.

Afin d'arriver à approfondir la nature de ces complexes sans avoir à les décomposer, il sera tout d'abord nécessaire d'en effectuer un fractionnement intensif, et en second lieu il sera d'une importance capitale que la substance protéique devant être soumise au fractionnement soit réellement pure, ou du moins aussi pure qu'on pourra l'obtenir sans qu'elle soit privée de ses propriétés caractéristiques.

C'est ici que nous rencontrons la seconde question que je

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<sup>1)</sup> Comptes-rendus du Laboratoire Carlsberg, 15, N° 11 (1923).

me suis proposé d'aborder aujourd'hui, à savoir la caractérisation individuelle des protéines. Comme cette question est en certains points étroitement liée à celle relative à la composition élémentaire des protéines, je vous demande la permission, Mesdames et Messieurs, de dire quelques mots sur cette dernière.

Comme chacun de vous le sait, les substances protéiques contiennent les éléments carbone, hydrogène, oxygène et azote, auxquels se joignent ordinairement de faibles doses de soufre et de phosphore. Les différentes protéines ont à peu près la même composition élémentaire, c'est vrai; mais néanmoins les différences, surtout sous le rapport de la teneur en azote, en soufre et en phosphore, sont si importantes, que pour la caractérisation de chaque groupe la composition centésimale nous fournit une aide précieuse. La teneur en soufre est très faible, celle en phosphore le plus souvent moindre encore. Il est donc tout naturel de se demander s'il s'agit ici de constituants véritables de la molécule même ou du complexe de molécules protéiques, ou bien si les composés contenant du soufre ou du phosphore ne sont liés à la protéine proprement dite que d'une façon peu solide, peut-être par adsorption, de manière que ces corps étrangers ne modifient pas essentiellement les propriétés caractéristiques de la protéine proprement dite. De semblables considérations se présentent tout naturellement quand les protéines en dissolution sont regardées comme composées d'une série de complexes plus ou moins étroitement unis dont les liaisons réciproques sont susceptibles d'être modifiées lorsqu'on fait varier la température, la concentration en ions hydrogène, la concentration en sels, etc., de la solution. Il serait trop long d'entrer ici dans un grand nombre de détails concernant ce problème; je devrai me borner, pour l'éclaircir quelque peu, à citer quelques exemples relatifs à la teneur en phosphore des protéines.

En faisant abstraction des nucléoprotéides et de leurs produits de décomposition, les acides nucléiques, qui occupent une position à part dans la chimie des protéines, il est à remarquer que la teneur en phosphore des protéines varie depuis des quantités tout à fait minimales jusqu'à 1 % du poids de la matière sèche. Parmi ces protéines, celles qui sont les plus riches en phosphore, tout en présentant par ailleurs des propriétés voisines ou identiques, ont été réunies en un groupe dénommé phosphoprotéines ou phosphoprotéides, dont le représentant principal est la caséine.

Pour ce qui regarde les autres protéines, on admet — implicitement, pour ainsi dire — que la teneur en phosphore provient d'impuretés, probablement de phosphatides adhérentes (lécithine et substances semblables). Selon moi, il est cependant douteux que cette question puisse être tranchée d'une manière aussi simpliste, et je vais citer quelques faits destinés à motiver ce doute.

La caséine ordinaire, préparée et purifiée soigneusement suivant le procédé de Hammarsten, contient environ 0.8 % P et env. 15.6 % N, donc environ 50 milligrammes de phosphore par gramme d'azote. On peut demander si toute cette quantité de phosphore fait partie intégrante de la molécule de caséine, ou bien si au moyen d'une purification ultérieure ou un fractionnement convenable de la caséine on pourrait diminuer sa teneur en phosphore sans lui faire perdre ses propriétés caractéristiques. Nous avons donc à faire ici la définition de la caséine, c'est-à-dire à rechercher comment on peut le mieux la caractériser. A cet effet, il paraît convenable de choisir la réaction bien connue utilisant la présure: par addition d'un sel calcique soluble et d'une solution de présure (extrait de la membrane muqueuse de la caillette de veau), la caséine se convertit en sel calcique insoluble de la paracaséine; c'est, comme vous le savez, une réaction spécifique, tout particulièrement appropriée à la caractérisation de la caséine. Comme je l'ai déjà dit, Linderstrom-Lang, du Laboratoire Carlsberg, a réussi par un fractionnement convenable, à partager la caséine en différentes fractions possédant des propriétés sensiblement différentes, notamment sous le rapport de la solubilité dans l'acide chlorhydrique renfermant du chlorure de sodium, — et, d'autre part, relativement à la teneur en phosphore. Certaines fractions contenaient un peu plus de 50 milligrammes par gramme d'azote, alors que d'autres n'en avaient pas même 20 mg., mais toutes les fractions donnaient la réaction si caractéristique avec la présure. Quelle est donc la teneur normale en phosphore de la caséine? Et, de plus, est-il possible de préparer une caséine dépourvue de phosphore et, malgré cela, capable de coaguler par la présure?

Si comme types des protéines pauvres en phosphore nous prenons la globuline et l'albumine ordinaires, provenant respectivement du blanc d'œuf aussi bien que du sérum du cheval, nous voyons que ces substances présentent une bigarrure extraordinaire, que j'ai reproduite à gros traits dans le tableau ci-dessous.

### Teneur en phosphore des albumines et des globulines.

	mg. P pour g. d'azote total		Solubilité dans des solutions de $\text{Am}_2\text{SO}_4$ dans des conditions données
	coa- gulable	précipitable par l'alcool	
Globuline d'œuf .....	2	—	—
Albumine d'œuf .....	7.5	{ Totalité de P. coagulable }	constant
Euglobuline ord. de sérum.	2—40	{ $\frac{1}{10}$ — $\frac{1}{50}$ du P. coagulable }	diminuant avec une te- neur croissante en P.
Albumine ord. de sérum...	0.3—1.5	{ $\frac{1}{20}$ — $\frac{1}{30}$ du P. coagulable }	croissant avec une te- neur croissante en P.
Albumine de sérum traitée à l'alcool-éther à $\div 4^0$	{ 0.04 }	{ la presque totalité de P. coagulable }	

Chacune des substances portées sur ce tableau a été bien purifiée par des précipitations et des dialyses répétées bien des fois ou, pour les albumines, par des cristallisations réitérées. Comme vous le voyez, parmi les protéines d'œuf l'albumine a une teneur en phosphore plus forte que celle de la globuline, alors que pour les protéines du sérum c'est le contraire. Dans l'ovalbumine, préparée et recristallisée de la manière habituelle, la teneur en phosphore se montre sensiblement constante et semble, relativement, assez fermement attaché au reste du complexe de molécules de l'ovalbumine, attendu que le complexe entier, y compris tout le phosphore, se précipite en même temps, soit que la précipitation soit effectuée par chauffage ou au moyen de l'alcool. Il en est tout autrement des protéines du sérum, où seulement une faible partie de la quantité de phosphore coagulable est précipitable par l'alcool. Il s'agit donc ici vraisemblablement — en plein accord avec ce qu'on a admis autrefois — de substances peu solidement liées aux complexes de protéines de sérum. Quant à la question de savoir si lesdites substances sont des impuretés ou s'il faut les regarder comme faisant partie du complexe d'albumine ou de globuline, elle ne saurait, je crois, être résolue qu'en examinant s'il est possible de les éliminer sans priver l'albumine et l'euglobuline de leurs propriétés caractéristiques. A mon avis, il importe surtout que l'albumine reste apte à cristalliser

— propriété que la moindre altération lui fait perdre —, et que l'euglobuline conserve sa propriété bien connue d'être difficilement soluble ou tout à fait insoluble dans l'eau, mais soluble dans des solutions salines faibles, d'où une addition d'eau peut la reprécipiter.

Or, il est heureux que W. B. Hardy et Mme S. Gardiner<sup>1)</sup> aient imaginé un procédé — utilisé plus tard par E. O. Young<sup>2)</sup> — permettant d'éliminer à peu près tout le phosphore coagulable sans faire perdre à l'albumine ou à la globuline leurs propriétés spécifiques ci-dessus indiquées; à cet effet, lesdits auteurs, ayant précipité le sérum à  $\div 4^{\circ}$  au moyen de l'alcool, le traitent à fond à cette même température avec l'alcool et l'éther. Ainsi donc, pour les sérum-protéines, il ne s'agit que d'impuretés, et non de parties intégrantes, et, par conséquent, le phosphore contenu dans ces protéines ne peut probablement être responsable de ce fait assez étrange, que dans de certaines conditions l'euglobuline devient d'autant plus difficilement soluble dans une solution de sulfate d'ammonium, que la teneur en phosphore est plus élevée, alors que pour l'albumine c'est le contraire; les causes doivent sans doute être cherchées ailleurs.

J'ai tenté de purifier l'albumine d'œuf, elle aussi, en la soumettant à  $\div 4^{\circ}$  et à des températures encore plus basses, au traitement par l'alcool et l'éther; mais on ne parvient pas à en extraire du phosphore: cette albumine se dénature complètement, et après la dénaturation elle contient comme auparavant  $7^{mg}.5$  P par gramme d'azote. Je puis ajouter qu'à la suite d'un traitement pareil, la caséine ne cède pas, elle non plus, de substances phosphorées à l'alcool.

Il paraît donc qu'il y a sous ce rapport une différence essentielle entre les deux albumines, en ce sens que celle du sérum doit être considérée comme dépourvue de phosphore, tandis que — du moins, jusqu'à plus ample informé — il faudra regarder celle des œufs comme en renfermant.

Dans quelques travaux publiés il y a une dizaine d'années au sujet de l'ovalbumine, nous avons cru pouvoir, nous appuyant sur des mesures de la pression osmotique de solutions de cette albumine, évaluer par une simple estimation approximative le poids

<sup>1)</sup> Journ. Physiol., **40**, 68 (1910).

<sup>2)</sup> Proc. Roy. Soc., Londres, **B. 93**, 15 (1922).

a l'état anhydre de la molécule ou du complexe moléculaire de l'ovalbumine à 34 000, avec 380 atomes d'azote. Or, cette grandeur moléculaire s'accorde très bien avec le poids moléculaire (33 200) calculé l'année dernière par E. J. Cohn<sup>1)</sup>, qui supposait la présence de deux groupes de tryptophane dans la molécule, et, d'autre part, elle concorde également avec le poids moléculaire 33 000 à 35 000) que I. B. Nichols, usant de la méthode de centrifugation imaginée par Svedberg, vient de trouver pour l'ovalbumine bien purifiée<sup>2)</sup>.

Si donc nous estimons à 34 000 le poids moléculaire et à 380 le nombre des atomes d'azote, un simple calcul donnera pour résultat qu'un atome de phosphore dans cette molécule demanderait une teneur de 5<sup>mg</sup>.8 de phosphore par gramme d'azote. Vous voyez que l'ordre de grandeur de la teneur en phosphore est juste; cependant, 7.5 est trop grand par rapport à 5.8 pour que les déterminations analytiques puissent donner lieu à des erreurs se rapprochant de cette grandeur. Nous avons ainsi été amené à essayer de différentes manières s'il ne serait pas possible de réduire la teneur en phosphore de l'ovalbumine sans priver cette dernière de la faculté de cristallisation qui la caractérise. J'ai déjà dit qu'on n'y réussit pas au moyen du traitement à l'alcool et à l'éther à basse température. Nous n'y sommes pas parvenu non plus par cristallisation fractionnée à des concentrations différentes: la teneur en phosphore de différentes fractions ne variait que de 7<sup>mg</sup>.52 à 7<sup>mg</sup>.78 P par gramme de N.

#### Teneur en phosphore de l'albumine d'oeuf.

	mg. P pour g. N
Albumine ordinaire .....	7.5
— fractionnée.....	7.52—7.78
Électrodialyse:	
Liquide anodique .....	ca. 8.5
— intérieur .....	7.0—5.0
Abandonnée dans de la glace .....	7.3—4.7
Calculé pour 1 P à 380 N .....	5.8

<sup>1)</sup> Physiol. Reviews, **V** N° 3, 359 (1925).

<sup>2)</sup> Zeitschr. physik. Chem., **121**, 76 (1926).



Nous avons obtenu de meilleurs résultats par électrodialyse, dans l'appareil bien connu de Pauli<sup>1)</sup>, de solutions d'ovalbumine pauvres en électrolytes. Nous avons employé deux membranes de collodion, entre lesquelles la solution d'ovalbumine fut placée. On s'assura que les membranes de collodion étaient bien impénétrables à l'ovalbumine lorsqu'il ne passait pas de courant par le liquide; mais sitôt le circuit fermé on voyait l'ovalbumine commencer à traverser la membrane à l'anode, tandis que le liquide avoisinant la cathode demeurait exempt d'albumine durant plusieurs jours. Je n'ai pas le temps d'entrer dans les détails de cette réaction assez singulière; je me bornerai à signaler que, par rapport à la teneur d'azote, nous avons constamment trouvé plus de phosphore dans le liquide anodique que dans le liquide intérieur restant. Pourvu qu'on eût soin de veiller à ce que les solutions d'ovalbumine soumises à l'électrolyse ne renferment que des doses infimes d'électrolytes, l'albumine du liquide anodique aussi bien que celle du liquide intérieur pouvaient être amenées à cristalliser, et après des recristallisations répétées la teneur en phosphore de l'albumine provenant du compartiment de l'anode se trouvait un peu plus élevée que la teneur normale (soit environ 8<sup>mg</sup>.5 P par gramme de N), tandis que l'albumine tirée du liquide intérieur montrait une proportion plus faible de phosphore (allant d'env. 7 à 5 milligr. env. de P par gramme de N).

Enfin, je dois mentionner que des solutions d'ovalbumine — surtout dialysées et partant pauvres en électrolytes —, quand on les laisse dans la glace à la glacière, saturées de toluène, laissent déposer au bout d'un certain temps une partie de l'albumine à l'état dénaturé. Si l'on sépare par filtration le dépôt, l'albumine restant dissoute montre des propriétés normales, sa pression osmotique est normale, elle cristallise facilement et promptement, de manière normale. Il a cependant maintenant été constaté que l'ovalbumine ainsi obtenue, recristallisée à plusieurs reprises, contient moins de phosphore que celle nouvellement préparée, et elle en contient d'autant moins qu'elle a été abandonnée à elle-même pendant plus longtemps. La teneur en phosphore des échantillons examinés a varié de 7<sup>mg</sup>.3 à 4<sup>mg</sup>.7 P par gr. N; cette dernière valeur se rapporte à une solution d'ovalbumine dialysée, qui avait séjourné durant 11—12 années dans la glace à la glacière et qui

<sup>1)</sup> Biochem. Zeitschr., **152**, 357 (1924).

malgré cela, après séparation du dépôt, s'est trouvée cristalliser facilement et très régulièrement. Vous voyez donc qu'il est bien possible de préparer de l'ovalbumine bien cristallisée ayant une teneur en phosphore anormale et notablement inférieure à la teneur ordinaire: pourtant, la proportion de phosphore est toujours d'un ordre de grandeur tout autre que dans l'albumine de sérum, et, comme je l'ai déjà dit, je suis toujours d'avis que dans la grande molécule d'ovalbumine il existe un seul groupe phosphoré.

Afin d'éviter tout malentendu, je dois vous dire en terminant que, bien entendu, je ne regarde point la faculté de cristallisation des albumines ou le pouvoir de réaction de la caséine envers la présure comme suffisant pour une caractérisation complète de ces protéines. J'ai récemment traité ce problème dans un petit mémoire<sup>1)</sup>, et je dois me contenter ici d'affirmer que lesdites propriétés, tout en étant des caractères distinctifs nécessaires, ne suffisent point pour caractériser les protéines dont il s'agit.

J'espère que l'exposé sommaire que je viens de vous donner de quelques-uns des problèmes captivants qui en ce moment fixent l'attention des chercheurs s'occupant de la chimie des protéines, aura contribué à vous montrer avec quelle persévérante énergie ils s'efforcent d'étendre les connaissances que nous possédons sur ces substances si importantes.

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<sup>1)</sup> S. P. L. Sørensen: Proteins, Lectures given in the United States of America in 1924 (The Fleischmann Laboratories).

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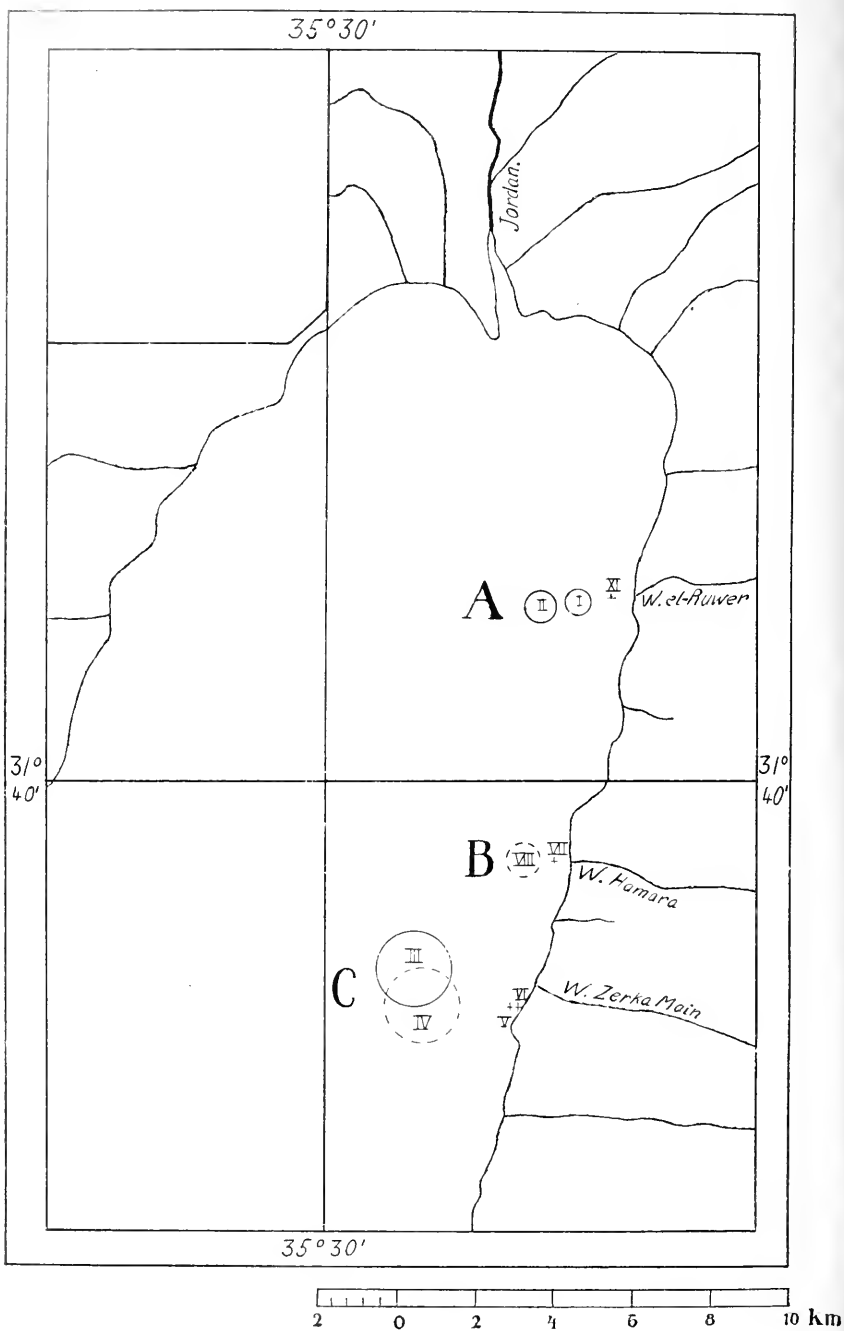
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Map showing northern part of the Dead Sea.

# AN ANALYTICAL INVESTIGATION OF WATER SAMPLES FROM THE DEAD SEA.

(MATERIAL FROM PROFESSOR, DR. LUDWIG BRÜHL'S EXPEDITION  
1911—1912.)

BY

R. KOEFOED AND G. HAUGAARD.

## PREFACE.

In the spring of 1911, Dr. Ludwig Brühl, now Custos and Professor of the "Institut und Museum für Meereskunde" in Berlin, informed Professor, Dr. S. P. L. Sorensen, Head of the Chemical Department of the Carlsberg Laboratory, that an Expedition was to be sent out at his, Professor Brühl's instigation, with economical support from the "Gesellschaft für Palastinaforschung" in Berlin, and from a prominent Berlin merchant, Hr. Oscar Tietz. The Expedition was to proceed to Palestine in the winter of 1911—1912, with a view to investigating, by the means now available, the little known, but doubtless peculiar and interesting hydrographical and chemical conditions of the Dead Sea.

Recalling the fact that important fundamental investigations in the sphere of modern hydrography had been carried out in Denmark and by Danish investigators — J. G. Forchhammer, Martin Knudsen and S. P. L. Sorensen etc. — Professor Brühl at the same time enquired whether it might be possible to persuade a Danish chemist to join the Expedition, which was to be under the leadership of Professor Brühl himself, and in connection with which a considerable amount of preliminary work had already been done. The funds available for carrying out the work however, did not suffice to cover the expense of the Danish chemist's participation. This being so, Professor Brühl, who had previously been connected with Denmark in various ways, and was well acquainted with Danish affairs, suggested that Professor

Sorensen might consider whether it would be possible for the Carlsberg Foundation to defray the cost of the Danish chemist's participation, and of the chemical equipment involved.

The Carlsberg Foundation approved of the plan, and mag. sc. Rudolph Koefoed, a former Brewery Director, was invited to join the Expedition, an invitation which Dir. Koefoed was very pleased to accept.

In addition, the geodetist Dr. Schoede, from Germany, also joined the Expedition.

At the close of October 1911, the Expedition set out from Berlin, arriving at Jaffa on Friday the 10th of November. Some time now elapsed in various negotiations. Much time was wasted in coming to terms with the owner of a motor boat on the Dead Sea. The vessel, which had been placed at the disposal of the Expedition by the owner, was found to have a defective motor; despite energetic protests on the part of Professor Brühl, and promises on the part of the owner, it proved impossible to get the boat to work. The Expedition was therefore obliged to make do with rowing boats, which caused very considerable delay, besides calling for more hands to work them.

On the 18th of December 1911, the Expedition camped at the mouth of the Jordan. The closing days of December were very stormy, and the actual taking of samples could not therefore be commenced until the 1st of January 1912 (Station I).

During the month of January, depth samples were obtained from various localities in the northern part of the lake (Stations I and II). It was intended to take samples throughout the entire extent of the lake, but the Expedition did not manage to get farther south than Zerka Main (Stations III, IV, V and VI) as various circumstances then compelled a return to the north. The time was then far advanced, and living by the Dead Sea proved very wearying indeed in the long run; it was however, not least the attitude of the rowers that weighed with the party in relinquishing further efforts towards the south.

On the way back, some further stations were taken (VII, VIII, IX, X, XI and XII), and the Expedition then entered on the return journey to Europe.

On reaching home, Director Koefoed commenced the chemical investigation of the material obtained, at the same time entering upon a thorough study of the literature. Unfortunately,



his work was hindered by illness, which for some periods put a stop to it altogether. Director Koefoed was therefore obliged to engage an assistant. His daughter, Froken Ella Koefoed, undertook the duties at first, and has carried out a large proportion of the analyses from Station III.

During the past few years, I have myself carried on the work first as Assistant to Director Koefoed, and later, after his death, independently. It is not without some regret that I conclude this work alone, knowing how keenly Director Koefoed was interested in it himself.

Finally, I beg to thank Professor, Dr. S. P. L. Sorensen very heartily for the assistance he has rendered me in the course of the work; I also wish to thank my friend, cand. polyt. Linderström-Lang, for much valuable advice.

GOTTFRED HAUGAARD.

## INTRODUCTION.

This work is divided into four sections.

The first gives an historical survey of previous analyses from the Dead Sea. The earliest analyses are described in detail, for historical reasons. The analytical results are worked up into tabular form. A graph in connection with the same gives rise to certain observations.

The second section gives a brief description of the methods of analysis. The electrometrical bromide determination method however, which is given in a new form, is described more in detail.

In the third section, the analytical results are given in tabular form. The results are noted in grams per 1000 g. of the water sample; as regards the quantity of oxygen and hydrogen sulphide however, in cc at  $0^{\circ}$  and 760 mm pressure. In the case of Station XI, the results are also given in grams and gram equivalents per 1000 cc at  $20^{\circ}$ .

Finally, the fourth section contains a discussion of the results.

## **I. Historical Survey of previous analyses of water samples from the Dead Sea.**

Analytical chemistry has from an early date concerned itself with the Dead Sea. We therefore find, in the literature, a number of analyses, but they do not agree very well one with another. The discrepancies are greatest in the case of the oldest analyses, which is naturally to be expected, seeing that analytical chemistry itself is not much older than the first analysis of water from the Dead Sea. These analyses illustrate one side of the history of analytical chemistry, though some of them are of more than merely historical interest.

The first analyses will be dealt with at some length, as we have thought it worth while to consider how people analyzed a hundred years ago. The analytical results will not be given in this connection, but are reproduced, in a somewhat different form, in the tables, together with the later analyses (Table 2). The list on p. 11 gives the works corresponding to the analyses in the table.

It was as a rule from pilgrims that the analysts obtained their samples, and it is therefore not surprising that information as to precise locality, depth and time is incomplete or altogether lacking. We may however, doubtless take it for granted that the great majority of the water samples were taken from quite near the surface, and the measurements of specific gravity are therefore of particular importance.

Maquer, Lavoisier and Lesage were the first to analyze a water sample from the Dead Sea. They communicated the result to the Academy in 1778. The water sample was exhibited in two well sealed bottles, and was clear, devoid of smell, with a piquant, bitter taste. One of the bottles contained a clump of crystals precipitated from the water, showing that it was saturated with salt. The specific gravity of the water sample as compared with distilled water was determined by means of a large floating weight with thin stem.

The process of analyses was as follows: By evaporation to half the volume, sodium chloride is produced, in regular crystals; these are filtered off, dried, and weighed; they contain however, as the writers also point out, calcium and magnesium chloride; moreover, the entire quantity of sodium chloride is not crystal-

lized out. The filtrate from the crystals is evaporated, dried and weighed. Distilled water is then added, and afterwards, little by little, sulphuric acid, whereby calcium sulphate is precipitated. Subsequent evaporation and heating gets rid of the hydrochloric acid, leaving a mixture of calcium-, magnesium- and sodium sulphate. The sulphates are weighed, water is added, and there remains the greater part of the calcium sulphate; it is filtered off, dried and weighed.

This method of analysis, as will easily be seen, cannot give reliable results.

In 1807, Alexander Marcet published an analysis effected by himself and Tennant of water from the Dead Sea. For the purpose of their analysis, they required to know the equivalent conditions in regard to calcium-, magnesium- and sodium chloride. These equivalents they ascertain with great accuracy in the following manner. They first determined, by calcination, the content of calcium oxide in marble. They then suspend a piece of marble in a glass of hydrochloric acid, until all the acid is bound. From the weight of the calcium chloride formed, which they find by evaporation of the liquid in the glass, and the loss of weight of the marble, they now ascertain an equivalent for calcium oxide in hydrochloric acid.

This marble method serves as the basis of all their analytical operations. The equivalent proportions in magnesium chloride for instance, are determined by dissolving a known quantity of magnesium oxide in a known quantity of hydrochloric acid (according to the marble method). The surplus hydrochloric acid is determined by marble.

The equivalent values for sodium chloride are found by precipitating a weighed quantity of sodium chloride with silver nitrate. The silver chloride (luna cornea) precipitated is melted and then weighed. The calculation demands a knowledge of the equivalent values in luna cornea, and a known quantity of hydrochloric acid is therefore precipitated with silver nitrate, and the weight of the silver chloride formed determined. Marcet's and Tennant's results are shown here in tabular form, compared with the corresponding figures calculated from the latest atomic weights.

The table clearly shows what exceedingly fine analysts Marcet and Tennant must have been.

TABLE I.

	Marcet & Tennant	Calculated from atomic weights 1925
100 g. marble.....	56.1 g. CaO	56.0 g. CaO
» CaCl <sub>2</sub> .....	50.77 g. »	50.51 g. »
» MgCl <sub>2</sub> .....	43.94 g. MgO	42.34 g. MgO
» NaCl .....	54 g. Na <sub>2</sub> O	53 g. Na <sub>2</sub> O
» AgCl .....	80.95 g. Ag <sub>2</sub> O	80.84 g. Ag <sub>2</sub> O

The method they employed in analyzing the water samples was as follows: The analysis was divided into two parts. In one part, the chloride content was determined by precipitation with silver nitrate. In the other, calcium was precipitated with ammonium oxalate, and in the filtrate from this, magnesium was precipitated with ammonium carbonate in ammonia water. The calcium oxalate was transformed into carbonate by heating, and a known quantity of hydrochloric acid added to excess. The surplus was determined by the marble method, whereby the content of calcium was ascertained. The magnesium carbonate was heated to glow heat, and the magnesium oxide determined by the marble process. They also determined the sulphate content. The content of sodium chloride was arrived at by calculation. Marcet and Tennant tested the applicability of these analysis methods with known solutions, and obtained good results.

Some years later, Klapproth examined a sample of water from the Dead Sea. Klapproth evaporated the water and extracted the residue with spirits of wine. This dissolved the calcium and magnesium chloride, while the greater part of the sodium chloride remained undissolved. After evaporating the alcohol and making a fresh extract with alcohol again, he got a further quantity of the sodium chloride separated off from the calcium and magnesium chlorides. The alcoholic solution was evaporated, and after dissolving in water, the calcium was precipitated with sulphuric acid, filtered off, and dried and weighed. The sum of the calcium and magnesium sulphates was also determined. From these operations, it is possible to arrive at the content of sodium, calcium and magnesium.

In 1819, Gay Lussac published the fourth analysis of a water sample from the Dead Sea. His sample was obtained in a tinned iron bottle, such as the Bedouin use for selling water to the pilgrims. Gay Lussac gives no details as to the method of his analysis.

In 1922, Hermbstaedt published an analysis. The two bottles used by Hermbstaedt for analysis were filled on the third of September 1819 about 10.000 ft. from the mouth of the Jordan, in a south-easterly direction. One of the bottles was filled close to the shore, the other 90 ft. from land. The water in both samples behaved alike. Hermbstaedt's method of analysis differs only in a few inessential points from that of Klapproth. Hermbstaedt found that the samples contained free hydrochloric acid. The free hydrochloric acid originates from the magnesium chloride, which is decomposed on heating, which fact escaped his attention.

Five years later, C. G. Gmelin published an investigation of a water sample from the Dead Sea. Gmelin included the bromine, discovered in 1826 by Balard, in his analysis.

In 1839, Apjohn published an analysis of water from the Dead Sea. The sample was taken in the rainy season, a quarter of an hour's march from the mouth of the Jordan, which explains the low specific gravity found by Apjohn.

In 1849, Marchand published an analysis. Marchand, in his paper, goes through the previous analyses and re-calculates them, giving the results in a table. He points out that the crystallisation method, though not a particularly accurate method of analysis, has nevertheless the advantage of showing »how the substances are interrelated one to another«.

The methods of analysis now become more uniform, and are less frequently described; they approach those we should use nowadays. We shall therefore also here conclude the description of the analytical processes employed.

Table 2 shows the analytical results recalculated. Only the chlorides of magnesium, calcium and sodium have been included; these salts occur in greatest quantity in the water of the Dead Sea. The calculation is made so as to show the quantity of chlorine, magnesium, calcium and sodium contained in 100 gr. of the mixed chlorides. This has been done with a view to rendering the analyses more easily comparable. The table is

not complete; we have not been able to include all the analyses; it does however, contain by far the greater number of the analyses published. One of the analyses which we found in the literature was, we considered, best omitted altogether. It was made by Boutron-Charlard and O. Henry<sup>1</sup>. These writers find a very low specific gravity and a high content of carbonates, which does not agree with other analyses either before or since, and we may therefore with good reason assume that the water sample was in reality from the river Jordan.

It still remains to be noted that Terreil's analyses, in 1866, are of a some what different character from the rest, Terreil's water samples having been procured from an expedition, the object of which was to explore the Dead Sea, so that his data as to depth, place and time are also good. The expedition in question was carried out in March—April 1864 under the leadership of the Duc de Luine. The samples, which were taken by Louis Lartet, are from different parts and different depths of the sea. Of the results of the expedition, only one surface sample, taken in the northern part of the sea, will be mentioned here. Some of the other results will be referred to in a later section.

The table shows, as might be expected, a certain uniformity in the analyses. The differences are often great; but we nevertheless find that nearly half the analyses (marked with an asterisk in the table) exhibit a magnesium content between 12.7 and 13.6. with a calcium content between 5.0 and 5.5. This might seem to suggest that little change has taken place in the composition of the surface water during the past hundred years.

C. Elschner's analysis gives a particularly low calcium and sodium content, and it might therefore be not unreasonable to suppose that his sample was procured, not from the Dead Sea itself, but from one of the salines along the shore.

The figures for specific gravity reveal an interesting course, best shown in a graph. In Fig. 1, the year of publication of the analysis — or date of the sample where known — is used as abscissa, with specific gravity as ordinate.

It should again be emphasised that the water samples were probably taken for by far the greater part from the surface water itself. The Bedouin, or the travellers in question, have simply

<sup>1</sup> Journal de pharmacie et de chimie **21**, 161 (1852).

TABLE 2.

Analyst	Year of Analysis	Date of sample	Measurement of specific gravity		100 gr. MgCl <sub>2</sub> + CaCl <sub>2</sub> + NaCl contains				Remarks
			Tempe- rature	Specific gravity	gr. Cl	gr. Mg	gr. Ca	gr. Na	
Maquer, Lavoisier og Lesage <sup>1)</sup> .	1778	.....	.....	1.24062	67	11	11	11	
Marcet & Tennant <sup>2)</sup> .	1807	.....	.....	1.211	66.9	10.7	5.8	16.6	
Klaproth <sup>3)</sup> .	1809	.....	.....	1.245	67.9	12.0	7.4	12.7	
Gay-Lussac <sup>4)</sup> .	1819	.....	17°	1.2283	69.2	14.9	5.5*	10.4	
Hernbstaedt <sup>5)</sup> .	1822	3. Sept. 1819	15° 6 C.	1.240	69.5	15.5	5.9	9.1	( Sample taken about 10,000 ft. S. E. ( from mouth of Jordan.
Gmelin <sup>6)</sup> .	1827	.....	16° 1/4	1.2122	68.6	13.6*	5.3*	12.5	( Sample taken in the rainy season, ( 1/4 hrs walk from mouth of Jordan.
Apjohn <sup>7)</sup> .	1839	.....	.....	1.153	66.9	10.7	5.0*	17.4	
Mareland <sup>8)</sup> .	1849	.....	.....	1.18415	68.4	13.5*	5.2*	12.9	
Moldenhauer <sup>9)</sup> .	1856	June 1854	.....	1.1160	67.9	12.9*	3.9	15.3	
Boussingault <sup>10)</sup> .	1856	8. May 1856	.....	1.194	68.4	13.2*	6.2	12.2	
F. A. Genth <sup>11)</sup> .	1859	1857	17° 5	1.1823	68.0	12.7*	5.1*	14.2	( Spec. gravity not measured by Roux, ( but calculated from his analysis.
Benjamin Roux <sup>12)</sup> .	1863	April 1862	20°	1.1704	68.2	13.0*	6.1	12.7	( Sample taken near the little island ( in the northern part of the lake.
Terreil <sup>13)</sup> .	1866	June 1864	.....	1.1647	68.5	13.9	5.4*	12.3	( Presumably a sample from one of ( the salines.
H. Fleck <sup>14)</sup> .	1881	.....	18° 8	1.186	68.3	13.3*	5.2*	13.2	Sample probably taken at the surface.
C. Elsner <sup>15)</sup> .	1900	.....	.....	.....	71.3	19.8	0.7	8.1	
Mitchell <sup>16)</sup> .	1901	.....	0°	1.203	67.2	10.9	6.1	15.8	
A. Stützer & Reich <sup>17)</sup> .	1907	March 1907	17° 5	1.1546	67.3	11.4	4.4	16.9	
R. Koefoed & G. Haugaard ...	1926	Jan. 1912	20°	1.151	68.2	13.2*	5.2*	13.4	Sample from Station XI.
A. Friedmann <sup>18)</sup> .	1912	9. June 1911	15°	1.1336	67.6	11.6	6.2	14.6	Sample taken "10 minut., row from the coffee house on the northern shore".
A. Heiduschka <sup>19)</sup> .	1914	.....	15°	1.159	—	—	—	—	No quantitative analysis made.

1) Memoires de l'Academie Royale des sciences, (1778).

2) Philosophical Transactions of the Royal society of London (1807).

3) Magazin der Gesellschaft naturforschender Freunde zu Berlin, 3. Jahrg. 139 (1809).

4) Annales de chimie et de physique 11, 195 (1819).

5) Jahrbuch der Chemie und Physik 4, 453 (1822).

6) Württemb. naturwissenschaftl. Abhandl. 1, 334 (1827).

7) Annales des mines (1839).

8) Journal für praktische Chemie 47 (1849).

9) Annalen der Chemie und Pharmacie 97, 357 (1856).

10) Comptes rendus 42, 1230 (1856).

11) Annalen der Chemie und Pharmacie 110, 246 (1859).

12) Comptes rendus 57 (1863).

13) Comptes rendus 62, 1329 (1866).

14) Chemische Centralblatt 427 (1881).

15) Chem. Zeitung 854 (1900).

16) Berg- und Huettenmännische Zeitung 225 (1901-02).

17) Chem. Zeitung 845 (1907).

18) Chem. Zeitung 147 (1912).

19) Süddeutsche Apotheker Zeitung (1914).

waded out a few steps into the sea, and filled an ordinary bottle with the water. Moreover, the samples must, generally speaking, be assumed to have been taken in the northern part of the lake, near the mouth of the Jordan, as this is the travellers' natural objective, and also the most easily accessible portion of the lake. According to Blanckenhorn<sup>1)</sup> the eastern and western shores

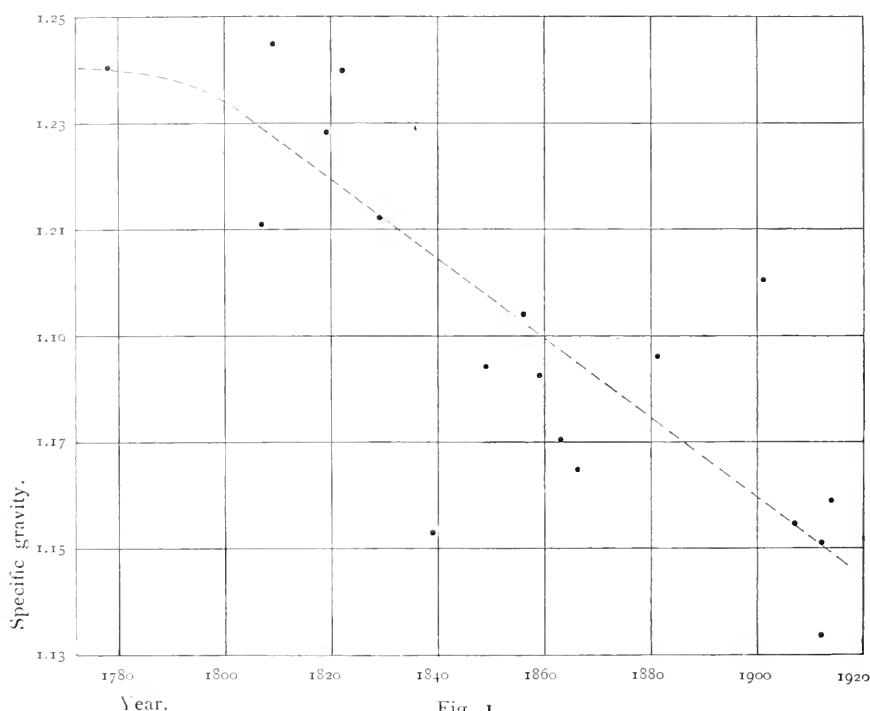


Fig. 1.

are extremely difficult of access, while the southern end of the lake forms a morass.

From these facts, then, it is evident that there must be some degree of relationship between the points in Fig. 1. It will be seen that the high values for specific gravity are almost exclusively confined to samples taken prior to 1840. There is only a single exception, from 1901. The dotted line, which has been drawn as a rough suggestion, indicates approximately the specific gravity of the surface water as a function of time.

It will be seen from the diagram that the specific gravity of

<sup>1</sup> Das tote Mer, p. 21, Berlin (1898).



the surface water has diminished in the course of the past century. This can only be due to a dilution of the water in the lake, which is to say, that the surface level of the Dead Sea must have risen. This result agrees both with direct observations and with the conclusions which can be drawn from the material afforded by the Brühl Expedition; this, however, will be further dealt with in a later section.

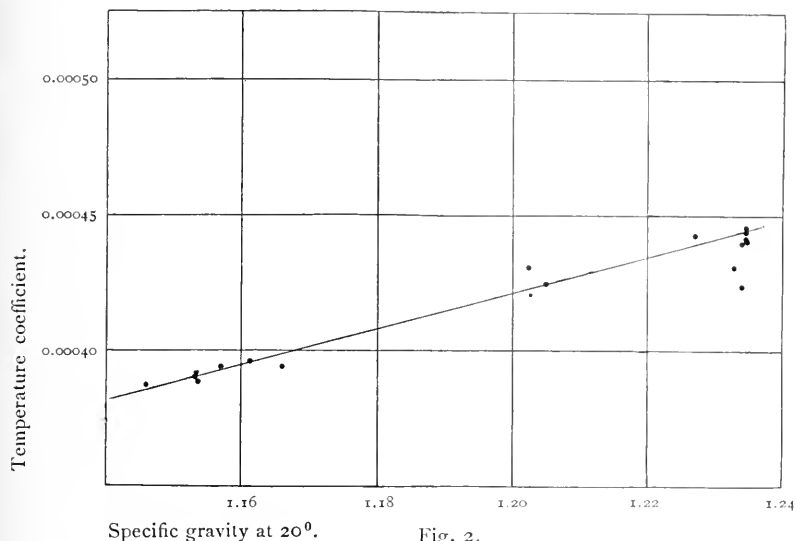


Fig. 2.

## II. Method employed in Quantitative Examination of the Water Samples.

The Water Samples were contained in green bottles, of 300 cc capacity; the bottles were completely filled, and well sealed with paraffined corks. Each sample consisted of two bottles. There were 210 bottles, thus representing 105 samples.

The quantitative examination consisted, firstly, of a measurement of the specific gravity, and secondly, of a quantitative analysis.

### A. Measurement of Specific Gravity.

The specific gravity at a certain accurately measured temperature, as compared with water at 4°, was determined for each sample. For this purpose, the Ostwald modification of the Sprengel pycnometer was used. The volume of the pycnometers was about 20 cc. Before setting the meniscus to the mark, the pycnometers

were left to stand for about 6 hours in a finely regulated thermostat at from 19 to 20 degrees. The drying of the pycnometers, weighing etc., were carried out with the greatest care, and correction made for upward drift. Double determinations were made throughout, and the difference between these amounted as a rule to only two or three in the fifth decimal point.

In the case of some of the samples, the specific gravity was measured at two temperatures, and in a very few cases, at three; these measurements were used for calculation of temperature coefficients. The temperature coefficient was found to increase with the specific gravity. Dependence on specific gravity is shown in Fig. 2. By means of the temperature coefficient, it was possible to refer all measurements of specific gravity to 20°00.

### *B. The Quantitative Analysis.*

It has been ascertained, from qualitative samples, that the water samples only contain any great quantity of the negative chloride, bromide and sulphate ions, and the positive sodium, calcium potassium and magnesium ions. The investigation as to the amounts of these ions in the water samples therefore falls naturally into a group by itself.

In addition to these, determinations were also made as to oxygen and hydrogen sulphide content; these, which were carried out in the course of the Expedition itself, will be described at the end of this section.

As regards the weighing of the samples, it was found unnecessary to weigh each sample separately; it was sufficient to weigh a large quantity in a tested measuring flask. After diluting with distilled water up to the mark, one could then, with a pipette, remove proportionate quantities for analysis. In testing the pipettes, it was found that pipettes of 20 cc and over will easily measure a cubic capacity with an accuracy of 0.1 %. The accuracy of the measuring flask was far superior to this, so that we might safely reckon with a degree of uncertainty in the measurement not exceeding about 0.1 % altogether, which is sufficient in consideration of the sources of error involved in these analyses.

The analytical results were worked out in grams per 1000 g of the water sample. Where no bromide determination was made, the silver equivalent value — i. e. the number of grams of silver equivalent to the halogen content — was determined. In the

case of Station XI, the analytical results were also worked out in equivalents per litre at 20°. Furthermore, a factor was calculated which permitted easy calculation of the results in equivalents per 1000 g H<sub>2</sub>O.

a) Determination of Silver Equivalent.

This determination was effected by titration with silver nitrate according to the Volhard method. In cases where the bromide content was determined, the chloride content was found as difference.

b) Bromide Determination.

Introductory remarks.

When a solution contains not only bromide ions, but also chloride ions, the determination of the bromide becomes the more difficult, the greater the concentration of chloride ions compared with that of the bromide ions.

The proportion between concentration of chloride ions and concentration of bromide ions in the Dead Sea is about 100. In this case, the gravimetric, indirect bromide determination is both extremely difficult and uncertain.

The same applies to Friedheim and Meyers method<sup>1)</sup>, which, as we know, consists in oxidizing the bromide ions, by means of a suitable medium, into free bromine, without affecting the chloride ions. The bromine is distilled over into a flask containing potassium iodide, the equivalent quantity of iodine is liberated, and then titrated with sodium thiosulphate.

Dir. Koefoed and Frk. Ella Koefoed have, for one of the stations, made a series of bromide determinations after Friedheim and Meyer, but they did not turn out altogether satisfactory, and I have therefore, at the suggestion of R. Koefoed, worked out an electrometric method of bromide determination. The well-known potentiometric method, described *inter alia* by Erich Müller<sup>2)</sup> and I. M. Kolthoff and N. Howell Furman<sup>3)</sup> is by no means good when there is anything like abundance of chloride ions present. The method will, however, be noted schematically here, partly because it was the observations which arise from a consideration of this which led to the method employed for bromide determination in the water samples.

<sup>1)</sup> Zeitschr. f. anorg. Chem. **1**, 407 (1892).

<sup>2)</sup> Die elektrometrische Maanalyse, Dresden and Leipzig (1921).

<sup>3)</sup> Potentiometric Titrations, New York (1926).

The method may be described as a titration method. We must first consider the case where no other halogen ions beyond the bromide are present. Titration is effected with a solution of silver nitrate, and between additions, the operator measures the potential of an element one half of which consists of the analysis liquid into which a silver electrode is dipped, the other half element being a normal electrode. The potential will vary in course of titration. The variation with addition of silver nitrate increases very greatly towards the close of the titration, that is to say, at the point where the concentration of silver ions equals

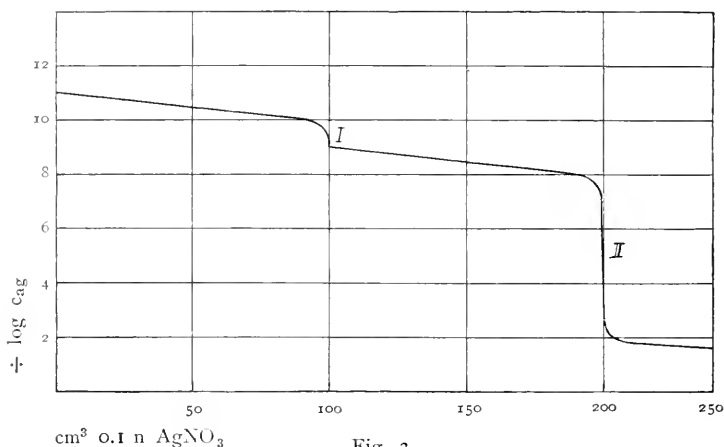


Fig. 3.

the concentration of bromide ions, and thereafter decreases. On plotting the course of the measurements as a graph, the point where the concentration of silver ions equals the concentration of bromide ions will be found to be a turning point, and here, the quantity of silver nitrate added is equivalent to the amount of bromide contained in the analysis.

If both chloride ions and bromide ions are present, and presuming that these are precipitated separately, so that the bromide of silver, which is less easily soluble, is precipitated first, then the potentiometric titration curve with bromide content equal to content of chloride, will assume the form shown in Fig. 3. In Fig. 3, the ordinate is  $\div \log c_{ag}$ , which magnitude, as we know, answers to the potential, and the abscissa is cc 0.1 n silver nitrate solution. The bromide and chloride content are here each individually equivalent to 100 cc 0.1 n silver nitrate solution. We

find two turning points, the first answering to the bromide content and the other to chloride content. It would then, if matters were as simple as all this, be easy to determine bromide together with chloride in this manner. F. W. Küster<sup>1)</sup> and A. Thiel<sup>2)</sup> have shown however, that chloride of silver and bromide of silver form permanent solutions, and it is easy to understand that this must have a levelling effect on the potentiometric titration curves, so that there will not be such sharp turning points, and the first of these turning points will not correspond directly to the bromide content.

An extract is given here from Küster's paper of 1899, showing the great importance of these permanent solutions of silver chloride and silver bromide both to the gravimetric and the potentiometric methods of analysis. The table gives the composition of the aqueous solution, which is in equilibrium therewith.

TABLE 3.

In the precipitate		In the solution	
AgBr	AgCl	KBr	KCl
3.25	6.64	1.41	1003
4.74	5.17	2.26	995
8.60	1.31	11.40	989
9.52	0.382	40.48	960
9.87	0.032	390.1	610

It will be seen that even the addition of a small amount of silver nitrate to a solution containing about a hundred times as many equivalents of chloride as of bromide will give a precipitate containing considerable quantities of silver chloride.

An attempt was made to explain the alteration in the composition of the precipitate during titration, and on this basis, to work out a method of analysis. This proved also partially successful; the method was, however, relinquished in favour of a simpler process which was at once found to give good results.

The method of bromide determination employed.

The principle here is as follows: the analysis is divided into two equal parts, A and B. Care is now taken, by chlorine treat-

<sup>1)</sup> Zeitschr. f. anorg. Chemie **19**, 81 (1899).      <sup>2)</sup> Ibid. **24**, 1 (1900).

ment. to free one half from bromide ions. To this half (B) is added such quantity of a potassium bromide solution that one may suppose there will be a little less bromide in this solution than in the other, A, whose bromide content is to be determined. From a potassium chloride solution as strong as the potassium bromide solution, add now, from a burette, to A as many cc as were added of the potassium bromide solution to B. A and B are then diluted to the same volume. We have thus obtained two solutions, of which the bromide concentration in one (B) is known, and presumably slightly less than that of the other (A). The halogen concentration is alike in both solutions, and they will also be entirely uniform in other respects. It should be noted, however, that the one solution (B) which has been treated with chlorine, may have altered its character if it contains substances capable of oxidation.

In cases where the concentration of bromide is of any importance, these two solutions will behave differently; e. g. if small quantities of silver nitrate be added, in equal amounts to each, the solutions will exhibit different concentrations of silver ions; there will therefore be a potential difference between a silver electrode placed in A and one placed in B, provided A and B are connected in the usual manner by an electrolyte bridge. By now increasing the quantity of bromide in B and at the same time adding the equivalent quantity of chloride to A in such a manner as to alter the volume of the solutions in the same degree, the potential difference will at first be reduced, and then, after passing zero, where A and B are alike, again increased, changing its sign at the same time. Since the bromide content of B is known throughout, the bromide content of A can now be ascertained. Before proceeding to the details of measurement, the chlorine treatment will here be described.

The liquid to be treated was poured into a Kjeldahl flask and some pieces of pumice stone added. Chlorine was then introduced into the liquid through a narrow tube. The chlorine was collected over saturated sodium chloride solution in a glass gasometer, and was rinsed with water in a bubbling flask before being introduced into the analysis. When the liquid was judged to have become saturated with chlorine, the chlorine and bromine were boiled out, and here the pumice stone proved of service, in preventing the liquid from frothing over. After cooling, the

chlorine treatment was repeated, and it was found necessary to repeat the process some six or seven times before one could be certain that all the bromine was driven out. For determination as to whether all the bromine had been driven out of the liquid, an extremely delicate and sure qualitative test was used, viz. that described by Richard Lorenz, E. Grau and E. Bergheimer<sup>1)</sup>.

The principle of their method is that the bromide ions are oxidised, by a suitable medium, to free bromine, which then affects a piece of paper prepared with a solution of fluorescein. The fluorescein was thus transformed into eosin, which makes itself apparent by its rosy tinge. The fluorescein paper was prepared by wetting soft filter paper with a solution of fluorescein in 35 parts of glacial acetic acid and 65 parts of distilled water, afterwards drying in the dark. The test may conveniently be carried out as follows: In a conical flask containing 65 cc, the sample is mixed with 5 cc saturated solution of potassium permanganate, and 0.2 cc concentrated sulphuric acid. The liquid is then diluted with water so as to leave only a little room in the flask. In a slit in a cork stopper, which must fit the flask accurately, is placed a small slip of the fluorescein paper. The whole is then set to stand in a dark place for a quarter of an hour. Any trace of bromine colours the paper a rosy red. In investigating the applicability of this method, it was found that bromide could still be distinctly shown to be present, even when there were 6000 times as many equivalents of chloride as of bromide, and the quantity of bromide answered only to 0.1 cc 0.1 n KBr. The test is thus unusually sharp.

Having obtained the solutions A and B<sup>2)</sup> they are placed one beside the other as shown in Fig. 4. A wooden slab with holes for the stirring rods (a), the liquid bridge (b) and the electrodes (c) is placed over the glasses, and attached by springs to the slab on which they stand. The stirring rods are of the well-known tulip type; their velocity must be fairly high (about 1000 revolutions per minute) in order to ensure the attainment of equilibrium within a reasonable time. The liquid bridge is filled

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<sup>1)</sup> Zeitschrift f. anorg. Chem. **136**, 90 (1924).

<sup>2)</sup> It should be noted that the half-element B can quite easily be obtained without chlorine treatment. For where it is only the bromide content of the sample which is unknown, it is easy to prepare a solution corresponding to B and which can then take the place of this.

with 3.5 n potassium chloride solution, and plugs of cotton wool are placed in the two openings of the tube.

The electrodes, of which there are two in each vessel, are strips of silver placed in rubber stoppers; each is soldered at the upper end to a piece of copper wire which again is soldered to a thicker piece. This can now easily be placed in a cup of mercury, and certain contact is thus easily obtainable, while the electrodes also can easily be changed.

The potential difference between the two half-elements was measured by the compensation method. The zero instrument was a mirror galvanometer, and the measuring bridge consisted of two rheostats.

As soon as the stirring rods and the rheostats have been set in position, the measuring can begin. Daylight was excluded during this process. The stirrers are set in motion, and an equal quantity of silver nitrate solution is then added from a pipette to each glass. The amount of silver nitrate added is as much as would be equivalent to about  $\frac{1}{10}$  to  $\frac{1}{5}$  of the bromide content. The object of this is to procure a halogen silver precipitate the composition of which should vary as little as possible during the measurement.

After the lapse of about 45 minutes, the electrodes are measured one against the other; as there are two electrodes in each of the vessels, this gives four measurements. The electrodes are taken up, dried, and changed over. After about 10 minutes, the electrodes are again measured one against the other. The mean of these measurements indicates with close approximation the electromotive force of the element, as the changing over partly eliminates the individual difference between the electrodes.

From two burettes, one containing a potassium bromide solution of known strength, the other a potassium chloride solution of like strength, there is now added to B a couple of cc of the bromide solution and to A an equal quantity of the potassium chloride solution. After about 45 minutes, the potential difference is again measured. This process is repeated until a suitable number of measurements have been obtained.

Three or four measurements have now been obtained, and if they lie near, or, preferably, on both sides of zero, it will be easy to find this graphically, and thus arrive at the bromide content of the analysis.



The degree of certainty obtainable by the electrometrical bromide determination method here described is best illustrated by the following table, showing the results of some determinations

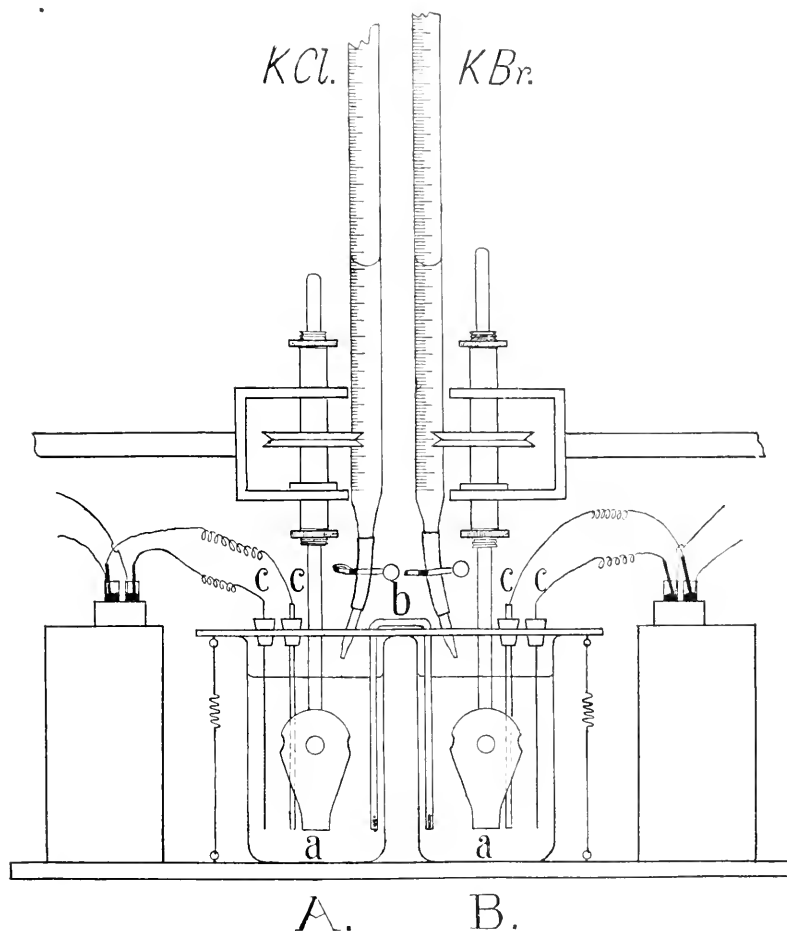


Fig. 4.

with solutions of precisely known composition. The remarks in the last column of the table refer to the preparation of B.

It will be seen from the table that the discrepancies in the area investigated, with the exception of some extreme values, lie between  $+3.0$  and  $-3.0\%$ .

TABLE 4.

Br/Cl	Bromide content of (A) in cc 0.05 n KBr solution	Found	Difference	Remarks
0.0397	70.0	70.4	+ 0.6 %	Not treated with chlorine.
0.0177	50.0	48.2	- 3.6 »	» » » »
0.0177	50.0	48.9	- 2.2 »	» » » »
0.0177	50.0	51.5	+ 3.0 »	Treated with chlorine.
0.0177	50.0	49.5	- 1.0 »	» » » »
0.0180	50.0	48.5	- 3.0 »	» » » »
0.0180	50.0	49.5	- 1.0 »	Not treated with chlorine.
0.0180	50.0	51.1	+ 2.2 »	Treated with chlorine.
0.0180	50.0	48.0	- 4.0 »	» » » »
0.0180	50.0	51.1	+ 2.2 »	» » » »
0.0180	50.0	47.8	- 4.4 »	» » » »
0.0405	40.0	39.6	- 1.0 »	» » » »
0.0405	40.0	40.0	0.0 »	» » » »

Finally, a single measurement of the bromide content in one of the water samples may be described in detail.

The sample was from Station III, and was taken at 2 metres depth. A and B contained each 50 cc of the sample. B was treated with chlorine as above described. To A was added 40 cc of a 0.05039 n potassium chloride solution, and to B 40 cc of a 0.05039 n potassium bromide solution. A and B were then diluted to 400 cc.

The further procedure was as previously described. About 45 minutes elapsed between each two measurements of the potential difference. The figures are given here in tabular form.

TABLE 5.

(B)'s content of 0.05039 n KBr solution	Potential in millivolts
40.00 cm <sup>3</sup>	+ 1.48
42.00 »	+ 0.24
44.00 »	- 0.78
46.00 »	- 1.88

Fig. 5 shows the results in graphical form; it will be seen that the bromide content in the water sample from Station III, 2 metres depth, was equivalent to the bromide content in 42.60 cc 0.05039 n KBr solution. That is to say, the water sample contains 0.0429 equivalents of bromine per litre, or 3.43 gr. per litre.

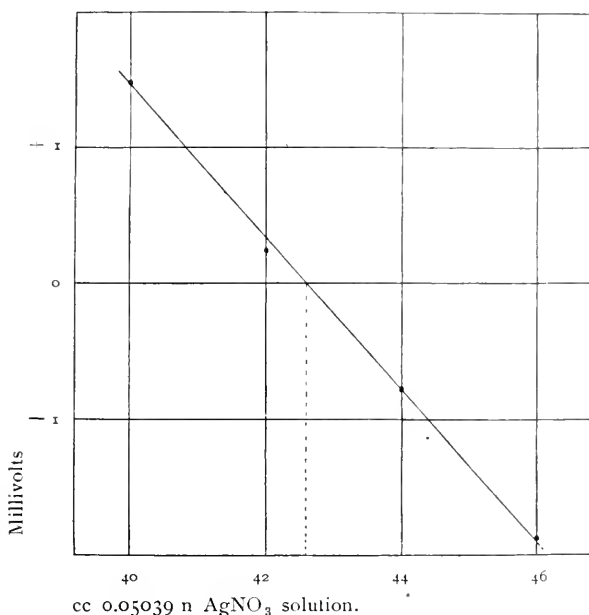


Fig. 5.

#### c) Sulphate determination.

The sulphate content was determined by precipitation of barium sulphate and weighing the same. The modification of this well-known analysis is due to Ernst Ruppig; it will suffice here to refer to Ruppig's paper<sup>1)</sup>.

#### d) Potassium determination.

Potassium was precipitated with hydrogen-platinum-chloride after a method indicated by H. Neubaur<sup>2)</sup>.

#### e) Calcium and Magnesium determination.

The calcium was precipitated with oxalic acid, care being taken to have abundance of ammonium salts present, so that no magnesium was precipitated<sup>3)</sup>. The calcium oxalate was left to

<sup>1)</sup> Wissenschaftliche Meeresuntersuchungen. Neue Folge. **14**.

<sup>2)</sup> Zeitsch. f. anal. Chem. **39**, 491—502 (1900).

<sup>3)</sup> Richards: Zeitschr. f. anorg. Chem. **28** (1901).

cool for 12 hours and then filtered off, washed and titrated with permanganate of potassium. In the filtrates from the calcium oxalate, magnesium was precipitated with sodium-ammonium-phosphate. After being left to stand for 24 hours at 50°, the precipitate was dissolved in hydrochloric acid and precipitated anew. The precipitate was now again left to stand at 50° for 24 hours, then filtered off in a platinum Gooch crucible, washed with 2.5 % ammonia water and heated to glow heat over a spirit blow lamp. The precipitate was thus transformed into pyrophosphate, and in this form it was weighed.

In order to ascertain whether the separation of calcium and magnesium was effective, some test determinations were made, which gave the following result: Out of 6 determinations, the deviation for calcium was 0.3 % average, greatest deviation 0.7 %. For magnesium, the mean deviation was 0.0 %, and the greatest deviation 0.5 %.

#### f) Sodium determination.

The sodium content was only determined indirectly, as the qualitative analysis has sufficiently clearly shown that the water samples, as mentioned on p. 14, only contain chloride, bromide and sulphate ions in any quantity, and also sodium, potassium, calcium and magnesium ions. As the content of the other ions is determined, the sodium content can be arrived at by a simple calculation. It is then easy to calculate the contents of salts in the water samples, reckoned, for instance, in grams per litre. These values thus calculated for the salt content agreed well with some directly found. These last will be described later on.

#### g) Determination of the »Amount of Salt«.

In some few of the water samples, the quantity of salt was determined. The method was that described by S. P. L. Sørensen<sup>1)</sup>, who defines the quantity of salt as the weight of soluble solids found in a kilo of sea water, all bromine being supposed to be replaced by chlorine, and all carbonate transformed into oxide; all organic matter, moreover, being burnt out. The definition is thus chosen, as it is possible to employ a method of salt determination which completely covers the definition.

Details of the method are given in the original paper; it will here suffice to describe the main features in the form in

<sup>1)</sup> Det kongelige danske Videnskabernes Selskabs Skrifter, 6. Række, 12. Bd., p. 115—134.

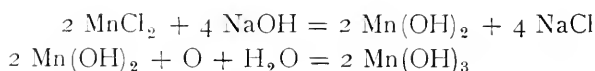
which the method was applied to the water samples. About 5 gr. of the water sample was weighed in a large porcelain crucible which had been previously weighed. Some drops of hydrochloric acid were added, and 5 cc of freshly prepared chlorine water which turned the liquid brown with the bromine thrown off. The crucible was then set to evaporate on a water bath. When the liquid had lost its colour, the addition of chlorine water was repeated and the liquid was then as a rule again coloured. This treatment had to be repeated several times, though it was not necessary to keep it up so long as with the bromide titration, as a slight trace of bromide is of no importance to the degree of accuracy desired in this case. When no more water could be evaporated on the water bath, the crucibles were placed in an electric drying apparatus, whereby further water was driven off. The temperature in the drying apparatus could not however, be brought to the point required to remove all the water, and transform the magnesium chloride to magnesium oxide. This was attained in a electric oven, where the temperature was about  $550^{\circ}$ . The crucibles were left to stand in this oven for about 8 hours, and were then, while still warm, placed each in its weighing glass. The stoppers of the weighing glasses had slits, so that the air pressures, in the subsequent evacuation in a vacuum dessicator, could be compensated. After the dessicator and thus also the weighing glasses with their contents, had been filled with dry air free from carbonic acid, the weighing glasses with contents were weighed. The operation was repeated to constant weight. This gives the weight of the water sample evaporated under these circumstances.

In order to determine the salt amount according to the above mentioned definition, it is necessary to know the amount of chlorine lost during evaporation in the form of HCl. The evaporated residue is therefore dissolved in dilute nitric acid, and the chloride content (b) in equivalents answering to 1 litre of the water sample determined. The halogen content (a) of the water sample prior to evaporation, also calculated in equivalents per litre, has been previously ascertained. This gives the amount of chlorine given off by heating. The correction term which must be added to the »salt amount« directly obtained, to get that previously defined, will be:

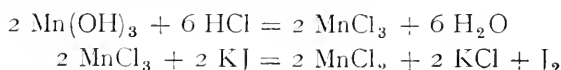
$$(a \div b) \cdot (Cl \div \frac{1}{2} O).$$

In addition to these analyses, determinations of oxygen and hydrogen sulphide were, as mentioned, also made. These fall somewhat outside the remaining determinations, as the quantity of oxygen and of hydrogen sulphide is so slight. The determinations were made in the course of the expedition by R. Koefoed.

h) The determination of oxygen was carried out according to Winkler's method<sup>1)</sup>. The principle is briefly as follows: On adding manganous chloride and sodium hydroxide to a bottle filled with oxygenised water, the following reactions will take place:

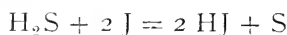


If now hydrochloric acid be added so as to dissolve the manganic hydroxide formed, and a potassium iodide solution then added, the following will take place:



The quantity of iodine evolved is titrated with sodium thiosulphate.

i) Determination of hydrogen sulphide was carried out according to Dupasquier-Fresenius<sup>2)</sup>. Acetic acid is added to hydrogen sulphide, and an iodine solution then added, whereupon the following reaction takes place:



The quantity of iodine not consumed is titrated with sodium thiosulphate.

This description of the method employed in the quantitative analyses has, with a single exception, been by no means exhaustive in detail; this would indeed have been superfluous. I have nevertheless thought it necessary to give this brief survey, for a proper appreciation of the analytical results.

Finally, it should here be mentioned that the bromine, and

<sup>1)</sup> A. Classen: *Ausgewählte Methoden der Analytischen Chemie* II, 41 (1903).

<sup>2)</sup> *Ibid.* II, 181 (1903).

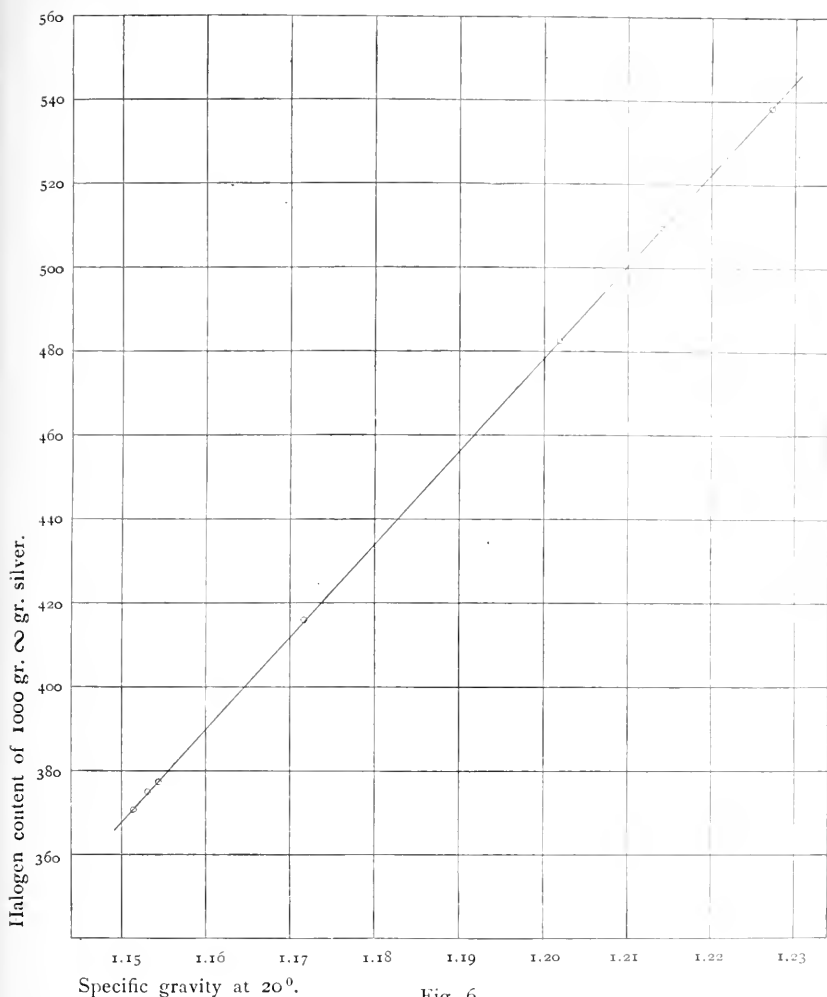


Fig. 6.

silver equivalent values, the calcium, magnesium and potassium determinations calculated in grams per 1000 gr. of the water sample from each station were found to be a linear function of the specific gravity. This rendered it possible to correct the analytical results, and also to reduce the number of analyses to some degree.

Fig. 6, 7 and 8 (see p. 36) illustrate this function in the case of Station XI. The abscissæ are the specific gravities at 20°, the ordinates being the silver equivalent, and the magnesium and calcium content respectively.

TABLE 6.  
**Station I.** 1. January 1912. Line Wadi Ghuwer—Frankenberg. (Area A).

Time	Distance from land in metres	Depth in metres	Temperature of water	Specific gravity at 20°	1000 gr. of the water sample contained						Remarks
					Halogens gr. silver	gr. SO <sub>4</sub>	gr. Mg	gr. Ca	gr. K	gr. Na	
2 <sup>26</sup> p. m.	700	Surface	17° 45	1.06570	169.7	0.350	10.22	4.22	1.88	11.06	No water sample taken. do.
3 <sup>26</sup> „	520	„	17° 7	—	—	—	—	—	—	—	
4 <sup>50</sup> „	700	„	16° 7	—	—	—	—	—	—	—	
4 <sup>05</sup> „	520	2	18° 0	1.15221	373.7	0.752	22.70	9.31	4.05	24.01	No water sample taken.
2 <sup>10</sup> „	700	5	18° 18	1.15299	375.4	0.722	22.82	9.35	4.07	24.08	
3 <sup>09</sup> „	580	10	18° 20	1.15321	376.0	0.737	22.84	9.36	4.08	24.20	
4 <sup>20</sup> „	530	15	18° 18	1.15344	376.4	0.740	22.90	9.37	4.09	24.10	No water sample taken.
4 <sup>52</sup> „	700	18	18° 10	1.15329	376.2	0.742	22.86	9.36	4.09	24.15	
5 <sup>05</sup> „	780	19	18° 08	—	—	—	—	—	—	—	
3 <sup>50</sup> „	480	20	16° 05	1.16027	391.4	0.726	23.95	9.77	4.34	24.70	Smells of hydrogen sulphide.
4 <sup>40</sup> „	610	25	17° 19	1.16523	402.4	0.721	24.74	10.04	4.47	25.19	Smells of hydrogen sulphide.
3 <sup>30</sup> „	520	30	19° 28	1.20399	488.8	0.556	30.78	12.16	5.71	28.96	Water rather dark in colour.
2 <sup>12</sup> „	700	50	20° 55	1.22602	537.8	0.404	34.24	13.36	6.42	30.96	do.



TABLE 7.  
Station II. 2. January 1912. Line Wadi Ghuwer—Frankenberg. (Area A).

Time	Distance from land in metres	Depth in metres	Temperature of water	Specific gravity at 20°	1000 gr. of the water sample contained					Remarks	1000 cc contains cc oxygen at 0° 700 mm
					Halogen	gr. SO <sub>4</sub>	gr. Mg	gr. Ca	gr. K	gr. Na	
955 a. m.	630	Surface	16° 8	—	—	—	—	—	—	—	1.53
1145 »	1100	»	18° 4	—	—	—	—	—	—	—	—
1250 p. m.	1080	»	18° 8	—	—	—	—	—	—	—	—
1045 a. m.	980	30	19° 0.1	1.20121	483.3	0.571	29.70	12.17	5.28	30.06	0.00
1037 »	1050	40	20° 0.18	1.21642	517.2	0.420	32.28	13.01	5.72	31.12	do.
1058 »	1010	50	20° 0.58	1.22797	540.8	0.373	34.33	13.63	6.14	31.30	do.
1116 »	1000	60	20° 0.67	1.23179	550.0	0.320	35.00	13.87	6.30	31.63	do.
1131 »	1050	70	20° 0.74	1.23260	551.6	0.312	35.19	13.88	6.28	31.56	—
1145 »	1100	80	20° 0.81	1.23283	552.4	0.283	35.31	13.90	6.27	31.49	0.00
1213 p. m.	1140	100	20° 0.92	1.23324	553.2	0.269	35.26	13.92	6.25	31.69	do.
1219 »	1170	120	20° 0.99	1.23350	553.8	—	35.19	13.95	6.23	31.86	do.
1237 »	1150	140	21° 0.12	1.23374	554.4	—	35.12	13.97	6.21	32.09	do.

TABLE 8.

Station XI. Wadi Ghuwer. 22. January 1912. Anchored at 77.5 m. (Area A).

Time	Depth in metres	Tempe- rature of water	Specific gravity at 20°	1000 gr. of the water sample contained					Remarks	1000 cc con- tains cc at 0°.	
				Halogen ∞ gr. silver	gr. SO <sub>4</sub>	gr. Mg	gr. Ca	gr. K	gr. Na	oxygen	hydrogen sulphide 760 mm.
8 <sup>30</sup> a. m.	Surface	9° 9'	—	—	—	—	—	—	—	3.58	0.32
8 <sup>30</sup> »	0.1		—	—	—	—	—	—	—	0.94	—
8 <sup>50</sup> »	1	16° 9.3	1.15136	371.4	0.728	22.93	9.01	4.11	23.39	0.99	0.30
9 <sup>01</sup> »	2	16° 5.6	1.15143	371.5	0.730	22.94	9.01	4.12	23.39	0.94	—
9 <sup>12</sup> »	4	16° 6.6	1.15310	375.2	0.732	23.20	9.11	4.16	23.55	0.82	—
9 <sup>23</sup> »	7	16° 7.1	1.15333	375.7	0.738	23.23	9.12	4.17	23.60	0.82	0.32
9 <sup>38</sup> »	10	16° 7.6	1.15317	375.4	0.740	23.21	9.11	4.16	23.60	0.82	0.24
9 <sup>48</sup> »	15	16° 9.3	1.15370	376.5	0.745	23.29	9.14	4.18	23.64	0.72	0.40
9 <sup>59</sup> »	18	17° 0.7	1.15391	377.0	0.750	23.32	9.15	4.18	23.67	0.80	0.29
10 <sup>10</sup> »	19	17° 0.1	1.15392	377.0	0.750	23.32	9.15	4.18	23.67	0.63	0.32
10 <sup>20</sup> »	20	17° 0.4	1.15435	378.0	0.752	23.39	9.18	4.20	23.71	0.56	0.34
10 <sup>30</sup> »	21	17° 1.4	1.15622	382.1	0.754	23.68	9.28	4.25	23.90	0.20	0.35

No water sample taken.

do.

Water sampler slightly open.

Faint smell of hydrogen sulphide.

do.

11113	24	17° 27	1.16471	400.8	0.748	24.99	9.77	4.48	24.68	Pronounced smell of hydrogen sulphide. Faint dark colour.	0.77
11124	25	17° 54	1.17159	416.0	0.710	26.04	10.16	4.66	25.37	Strong smell of hydrogen sulphide. Colour darker.	1.22
11135	26	18° 19	1.18581	447.3	0.670	28.23	10.97	5.05	26.70	do.	1.23
11153	27	18° 65	1.19708	472.1	0.635	29.97	11.61	5.35	27.78	do.	1.22
11204 p. m.	28	18° 82	1.19962	477.7	0.613	30.36	11.76	5.42	28.01	do.	1.13
11215	29	18° 93	1.20058	479.8	0.600	30.51	11.81	5.45	28.15	do.	1.00
11225	30	19° 00	1.20182	482.6	0.590	30.70	11.88	5.48	28.24	do.	1.06
11250	31	19° 22	1.20391	487.2	0.585	31.02	12.00	5.54	28.43	do.	1.06
130	32	19° 36	1.20633	492.5	0.575	31.39	12.14	5.60	28.66	Maximum of colouring. Faint smell.	1.08
100	35	19° 85	1.21407	509.5	0.505	32.59	12.58	5.81	29.37	do. do.	0.83
112	40	20° 29	1.21871	519.8	0.475	33.30	12.85	5.94	29.83	do. Very faint smell.	0.51
150	50	20° 48	1.22736	538.8	0.445	34.63	13.34	6.17	30.64	do. Trace of smell.	0.47
200	60	20° 68	—	—	—	—	—	—	—	Colour decreasing. No water sample taken. Hardly any trace of smell.	0.33
212	65	20° 73	—	—	—	—	—	—	—	Colour decreasing. No water sample taken. No smell.	—
225	68	20° 76	—	—	—	—	—	—	—	No water sample taken. Trace of colour.	0.22

Depth in metres	Specific gravity at 20°	1 Litre at 20° contains gram equivalents							
		Cl <sup>÷</sup>	Br <sup>÷</sup>	$\frac{1}{2}$ SO <sub>4</sub> <sup>÷÷</sup>	Sum of negative equi- valents	$\frac{1}{2}$ Mg <sup>++</sup>	$\frac{1}{2}$ Ca <sup>++</sup>	K <sup>+</sup>	Na <sup>+</sup>
1	1.15136	3.921	0.0427	0.0174	3.981	2.171	0.518	0.121	1.17
2	1.15143	3.923	0.0427	0.0175	3.984	2.172	0.523	0.121	1.16
4	1.15310	3.968	0.0432	0.0176	4.029	2.200	0.524	0.123	1.18
7	1.15333	3.975	0.0432	0.0177	4.036	2.203	0.525	0.123	1.18
10	1.15317	3.970	0.0432	0.0178	4.031	2.202	0.525	0.123	1.18
15	1.15370	3.984	0.0432	0.0179	4.045	2.210	0.526	0.123	1.18
18	1.15391	3.989	0.0433	0.0180	4.050	2.213	0.527	0.123	1.18
19	1.15392	3.989	0.0433	0.0180	4.050	2.213	0.527	0.123	1.18
20	1.15435	4.001	0.0434	0.0181	4.062	2.220	0.529	0.124	1.18
21	1.15622	4.051	0.0438	0.0182	4.113	2.252	0.536	0.126	1.19
22	1.15696	4.071	0.0440	0.0182	4.133	2.263	0.539	0.126	1.20
24	1.16471	4.281	0.0459	0.0181	4.345	2.394	0.568	0.134	1.24
25	1.17159	4.469	0.0475	0.0173	4.534	2.509	0.594	0.140	1.29
26	1.18581	4.865	0.0512	0.0165	4.933	2.753	0.649	0.153	1.37
27	1.19708	5.185	0.0539	0.0158	5.255	2.951	0.694	0.164	1.44
28	1.19962	5.257	0.0547	0.0153	5.327	2.995	0.704	0.166	1.46
29	1.20058	5.285	0.0547	0.0150	5.355	3.012	0.708	0.167	1.46
30	1.20182	5.322	0.0550	0.0148	5.392	3.035	0.713	0.169	1.47
31	1.20391	5.381	0.0555	0.0147	5.452	3.072	0.721	0.171	1.48
32	1.20633	5.450	0.0562	0.0144	5.520	3.114	0.731	0.173	1.50
35	1.21407	5.676	0.0590	0.0128	5.748	3.254	0.762	0.180	1.55
40	1.21871	5.813	0.0593	0.0121	5.884	3.337	0.782	0.185	1.58
50	1.22737	6.069	0.0614	0.0114	6.141	3.495	0.817	0.194	1.63

A is a factor whereby the results in the table must be multiplied to give the equiv

## Station XI.

1 Litre at 20° contains grams									A (see foot- note)	Depth in metres
Cl	Br	SO <sub>4</sub>	Mg	Ca	K	Na	Salts	H <sub>2</sub> O		
39.06	3.41	0.838	26.40	10.37	4.73	26.93	211.74	939.62	1.064	1
39.10	3.41	0.841	26.41	10.48	4.74	26.86	211.84	939.59	1.064	2
40.70	3.45	0.844	26.75	10.50	4.80	27.19	214.23	938.87	1.065	4
40.94	3.45	0.851	26.79	10.52	4.81	27.26	214.62	938.71	1.065	7
40.79	3.45	0.853	26.77	10.51	4.80	27.16	214.33	938.84	1.065	10
41.26	3.45	0.860	26.87	10.54	4.82	27.28	215.08	938.62	1.065	15
41.46	3.46	0.865	26.91	10.56	4.82	27.30	215.38	938.53	1.065	18
41.46	3.46	0.865	26.91	10.56	4.82	27.30	215.38	938.54	1.065	19
41.87	3.47	0.868	27.00	10.60	4.85	27.35	216.01	938.34	1.066	20
43.66	3.50	0.872	27.38	10.73	4.91	27.58	218.63	937.59	1.067	21
44.37	3.52	0.874	27.52	10.79	4.94	27.72	219.73	937.23	1.067	22
51.82	3.67	0.871	29.11	11.38	5.22	28.73	230.80	933.91	1.071	24
58.49	3.80	0.832	30.51	11.90	5.46	29.69	240.68	930.91	1.074	25
72.52	4.09	0.794	33.48	13.00	5.99	31.69	261.56	924.25	1.082	26
83.85	4.31	0.760	35.88	13.90	6.40	33.26	278.36	918.72	1.088	27
86.41	4.37	0.735	36.42	14.11	6.50	33.63	282.18	917.44	1.090	28
87.40	4.37	0.720	36.63	14.18	6.54	33.76	283.60	916.98	1.091	29
88.71	4.40	0.709	36.90	14.28	6.59	33.93	285.52	916.30	1.091	30
90.83	4.44	0.704	37.35	14.45	6.67	34.22	288.66	915.25	1.093	31
93.27	4.49	0.694	37.87	14.64	6.76	34.55	292.27	914.06	1.094	32
101.27	4.72	0.613	39.57	15.27	7.05	35.70	304.19	909.88	1.099	35
106.13	4.74	0.579	40.58	15.66	7.24	36.34	311.27	907.44	1.102	40
115.22	4.91	0.546	42.50	16.37	7.57	37.61	324.73	902.64	1.108	50

es per 1000 gr. H<sub>2</sub>O.

TABLE 10.

## Station VII. Wadi Hammarah. 17. January 1912. Anchored at 83 m. (Area B).

Time	Depth in metres	Temperature of water	Specific gravity at 20°	1000 gr. contains			Remarks	1000 cc at 20° contains	
				Halogens gr. silver	gr. Mg	gr. Ca		cc oxygen 0°, 760 mm.	cm <sup>3</sup> H <sub>2</sub> S 0°, 760 mm.
1135 a. m.	Surface	16° 9	1.15280	375.3	22.87	9.27	Just at surface.	—	
1223 p. m.	1	16° 8.5	1.15155	372.6	22.67	9.20	1 cm below surface.	0.96	
1212 "	2	16° 7.0	1.15209	373.8	22.76	9.23	.....	0.96	
1201 "	4	16° 7.2	1.15228	374.2	22.79	9.24	.....	0.97	
1150 a. m.	7	17° 0.00	1.15243	374.5	22.81	9.25	.....	0.91	
1138 "	10	17° 0.00	1.15298	375.7	22.90	9.28	.....	0.83	
1127 "	15	17° 0.04	1.15322	376.2	22.94	9.29	.....	0.86	
1116 "	18	17° 0.07	1.15387	377.6	23.04	9.32	.....	0.80	0.15
1105 "	19	17° 0.13	1.15409	378.0	23.07	9.33	.....	0.59	0.14
926 "	20	17° 0.12	1.15255	380.5	23.26	9.39	Water samples clear and without smell of hydrogen sulphide.	0.43	0.22
1038 "	21	17° 0.10	1.15727	384.8	23.58	9.50	Faint smell. Colourless.	Trace	0.31
938 "	22	17° 0.11	1.15914	388.8	23.87	9.60	do.	0.00	0.47
1050 "	23	17° 0.25	1.16080	392.3	24.19	9.68	Stronger smell. No colour.	"	0.51
949 "	24	17° 0.57	1.16693	403.4	24.97	9.95	Distinct smell and colour.	"	0.75
1003 "	25	18° 0.06	1.17593	422.6	26.39	10.42	Strong smell. Distinct colour.	"	1.31
1015 "	26	18° 0.22	1.18396	441.5	27.80	10.88	do.	"	1.28
1006 "	27	18° 0.50	1.18817	450.5	28.47	11.10	do.	"	1.17
133 p. m.	40	20° 0.30	1.19502	465.0	29.55	11.45	Distinct smell. Colour.	"	1.14
122 "	50	20° 0.56	1.21966	517.4	33.46	12.37	do.	"	0.48
1231 "	60	20° 0.66	1.22758	534.3	34.71	13.14	Fainter smell. Colour.	"	0.30
1247 "	65	20° 0.71	1.23131	542.2	35.30	13.33	Faint smell. No colour.	"	0.09
1257 "	67	20° 0.71	1.23244	544.5	35.47	13.39	do.	"	0.09
1300 "	70	20° 0.71	1.23255	544.7	35.48	13.39	Without smell or colour.	"	0.06

TABLE 11.

**Station VIII.** 17. January 1912. (Area B).

Time	Depth in metres	Tempe- rature of water	Specific gravity at 20°	1000 cc of water sample contains			Remarks
				Halogen ∞ gr. silver	gr. Mg.	gr. Ca	
3 <sup>38</sup> p.m.	Surface	17° 04	—	—	—	—	No water sample taken.
4 <sup>19</sup> "	200	21° 24	1.23322	551.7	35.98	13.49	
4 <sup>00</sup> "	225	21° 26	1.23327	551.8	36.02	13.47	
3 <sup>40</sup> "	256	21° 28	1.23343	552.2	36.07	13.36	
5 <sup>00</sup> "	277	21° 28	1.23334	551.9	36.01	13.47	Touched bottom at 270.5 metres.

**III. The analytical results.**

In the foregoing pages, the results of the analyses are given in tabular form. The position of the stations is indicated by circles or crosses on the chart p. 2. Where information as to the stations was rather deficient, the circles are dotted. It should be understood however, that divergence from the area indicated is in any case but slight. As regards those stations where the boat was anchored, the anchorage is marked with a cross.

The tables give, in addition to place and time, analytical results etc., also a column containing inter alia notes of such qualities in the water samples as smell and appearance. These observations are from the journal kept by R. Koefoed during the expedition; they are of particular importance where determination of hydrogen sulphide is lacking, and may here serve to some extent instead.

The tables are not given in the order in which the stations were taken, but in that order in which they naturally belong. Stations I, II and XI for instance, lie in an area marked A on the chart. Stations VII and VIII lie in an area marked B, while III, IV, V and VI which are the southernmost, lie in the area marked C.

Stations IX, X and XII are not marked on the chart. From these three stations there were only two samples (bottom samples). These samples will be dealt with in the next section. At the close of the present section, an analysis of a water sample from the river Jordan is given.

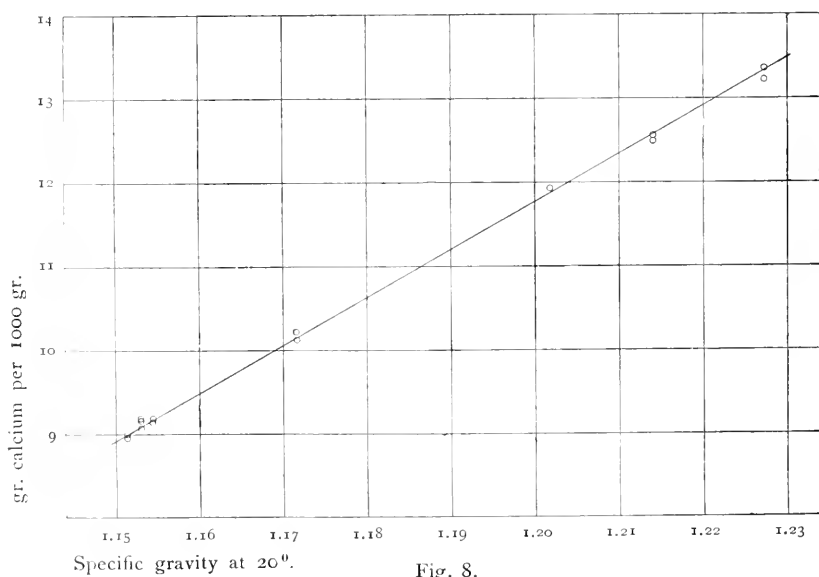
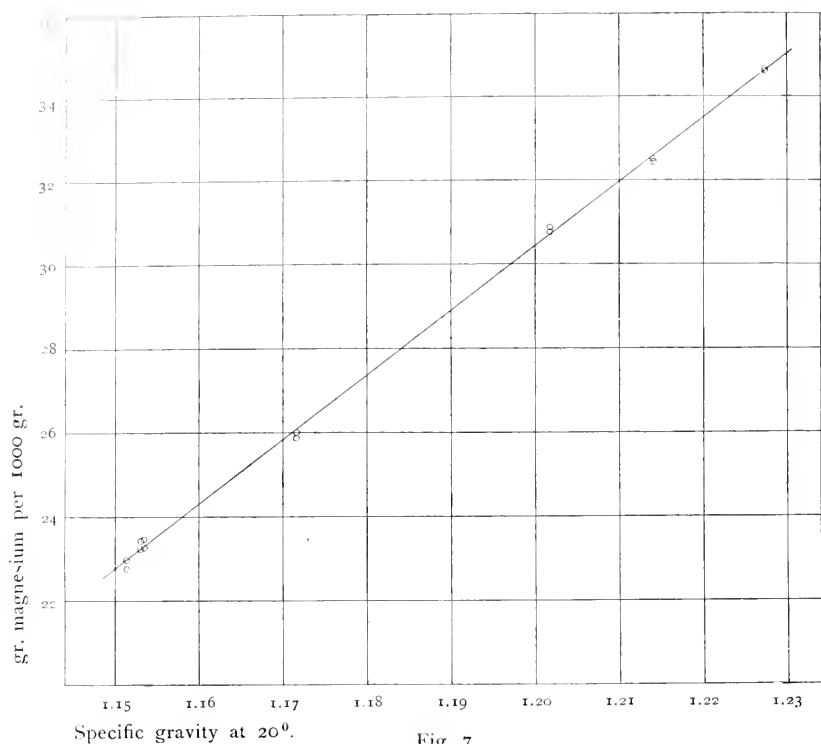




TABLE 12.  
Station III. 8. January 1912. (Area C).

Time	Distance from land in metres	Depth in metres	Temperature of water	Specific gravity at 20°	1000 gr. of the water sample contained					Remarks	1000 cc contains cc oxygen at 0° and 760 mm
					Halogen ∞ gr. silver	gr. SO <sub>4</sub>	gr. Mg	gr. Ca	gr. K	gr. Na	
1020 a. m.	1020	Surface	17°·2	1.154 <sup>1)</sup> (22°)	—	—	—	—	—	—	1.04
1025 »	—	»	17°·2	1.153 <sup>1)</sup> (17.5°)	—	—	—	—	—	—	—
1255 p. m.	1680	»	18°·4	—	—	—	—	—	—	—	—
215 »	—	»	18°·7	—	—	—	—	—	—	—	—
1050 a. m.	—	2	17°·23	1.15310	374.2	0.740	23.02	9.88	4.15	22.82	1.03
1109 »	1219	10	17°·40	1.15328	374.8	0.736	23.06	9.90	4.15	22.86	—
1127 »	1555	20	17°·14	1.15670	382.2	0.736	23.56	10.60	4.24	22.59	—
1143 »	1550	30	19°·03	1.20190	483.2	0.601	30.52	12.44	5.41	28.13	—
1208 p. m.	1680	50	20°·52	1.22738	538.8	0.413	34.44	13.76	6.07	30.59	0.00
1229 »	1570	70	20°·74	1.23210	549.6	0.360	35.22	14.03	6.20	31.00	—
1252 »	1680	100	20°·91	1.23321	551.8	0.349	35.38	14.09	6.23	31.10	—
113 »	1910	120	21°·02	1.23345	552.8	0.340	35.44	14.11	6.24	31.10	—
133 »	2000	150	21°·16	1.23402	553.8	0.334	35.52	14.13	6.26	31.17	—
203 »	1900	200	21°·21	1.23410	553.8	0.326	35.52	14.13	6.26	31.17	—

<sup>1)</sup> Specific gravity measured with Westphal's balance.

From this station we have some bromide determinations according to the electrometric method described in Section 2. The results of the measurements are not shown in this table, but are given in Section 5, where they can be read from Fig. 13.

TABLE 13.

## Station IV. Hamman Zara. 11. January 1912. (Area C).

Time	Depth in metres	Tempe- rature of water	Specific gravity at 20°	1000 gr. of the water sample contained					Remarks	1000 cc contains cc oxygen at 0° and 760 mm
				Halogen ∞ gr. silver	gr. SO <sub>4</sub>	gr. Mg	gr. Ca	gr. K	gr. Na	
1 <sup>49</sup> p.m.	Surface	17° 8	1.14615	360.4	0.725	22.04	8.82	3.86	23.09	Sample taken 1 to 2 cm below surface. 1.04
3 <sup>47</sup> »	do.	17° 8	1.14529	358.1	0.717	21.94	8.76	3.83	23.14	Sample skimmed just at the surface. 1.28
4 <sup>40</sup> »	do.	17° 6	—	—	—	—	—	—	—	do. No water sample taken.
4 <sup>40</sup> »	10	17° 10	1.15267	376.2	0.729	23.00	9.20	4.07	24.08	..... 1.00
4 <sup>25</sup> »	150	21° 19	1.23303	553.5	0.288	35.49	13.64	6.33	31.63	
3 <sup>07</sup> »	200	21° 23	1.23366	553.8	0.273	35.56	13.67	6.22	31.63	
3 <sup>26</sup> »	225	21° 25	1.23355	553.8	0.275	35.84	13.67	6.25	31.07	
2 <sup>46</sup> »	250	21° 28	1.23393	554.3	0.305	35.71	13.78	6.42	31.07	
4 <sup>06</sup> »	275	21° 28	1.23361	553.2	0.289	35.70	13.80	6.19	31.07	
2 <sup>21</sup> »	300	21° 28	1.23381	554.6	0.290	35.79	13.74	6.39	31.14	

TABLE 14.  
**Station V.** Hamman Zara. 12. January 1912. Anchored at 64 m. (Area C).

Time	Depth in metres	Tempe- rature of water	Specific gravity at 20°	1000 gr. of the water sample contained						Remarks
				Halogen ∞ gr. silver	gr. SO <sub>4</sub>	gr. Mg	gr. Ca	gr. K	gr. Na	
1104 a. m.	1	17° 03	1.15256	374.2	0.743	23.06	9.28	4.17	23.41	
1115 „	2	17° 04	1.15294	375.0	0.743	23.12	9.31	4.18	23.41	
1126 „	3	17° 11	1.15277	374.5	0.746	23.10	9.30	4.17	23.39	
1135 „	4	17° 12	1.15317	375.6	0.743	23.16	9.32	4.19	23.44	
1145 „	5	17° 06	1.15293	375.0	0.741	23.12	9.30	4.18	23.44	
1155 „	10	17° 06	1.15472	379.0	0.740	23.40	9.43	4.23	23.58	
1250 p. m.	20	17° 12	1.15754	385.0	0.732	23.82	9.56	4.30	23.87	
334 „	24	17° 26	—	—	—	—	—	—	—	Faint smell of hydrogen sulphide. No water sample taken.
1237 „	25	17° 38	1.16932	411.6	0.707	25.62	10.22	4.62	25.21	Distinct smell of hydrogen sulphide.
235 „	26	18° 53	—	—	—	—	—	—	—	Strong smell of hydrogen sulphide. No water sample taken.
214 „	28	19° 04	—	—	—	—	—	—	—	do.
1248 „	30	19° 14	1.20263	485.8	0.596	30.68	12.04	5.50	28.75	do.
203 „	35	19° 52	1.21381	511.0	0.500	32.40	12.66	5.80	29.95	do.
100 „	40	20° 23	1.21786	519.8	0.411	33.00	12.87	5.91	30.38	do.
112 „	50	20° 54	1.22622	538.4	0.370	34.26	13.34	6.13	31.23	Smell of hydrogen sulphide.
133 „	60	20° 06	1.23140	550.0	0.367	35.04	13.62	6.26	31.86	do.

TABLE 15.

**Station VI.** Hamman Zara. 13. January 1912.  
Anchored at 40 metres. (Area C).

Time	Depth in metres	Tempe- rature of water	Specific gravity at 20°	1000 gr. of water sample contained			Remarks
				Halogen ∞ gr. silver	gr. Mg	gr. Ca	
3 <sup>50</sup> p.m.	Surface	17° 30	—	—	—	—	No water sample taken.
3 <sup>57</sup> »	10	16° 99	1.15198	373.0	23.10	8.87	
4 <sup>12</sup> »	15	17° 08	1.15284	374.9	23.14	9.14	

*Analysis of the water sample from the Jordan.*

A water sample from the Jordan was procured at a spot just above the baptismal site. Quantitative investigation of this sample gave the following result:

Specific gravity at 19° 6: 0.99931

1 Litre contained:

0.516 gr. Cl  
0.117 gr. SO<sub>4</sub>  
0.356 gr. CO<sub>3</sub>  
0.100 gr. Ca  
0.065 gr. Mg  
0.425 gr. Na

in all, about 1.579 gr. salts per litre.

Qualitative tests for bromine and potassium gave positive results. Owing to the relatively small quantity available for analysis, the quantitative values are not particularly certain.

#### IV. Discussion of the Results Obtained.

a. Does the salt content at one and the same spot in the lake vary periodically during the 24 hours?

From the tables in the first section it will be seen that water samples from the same depth, but from different parts of the lake, are, roughly speaking, of the same composition. The differences found are comparatively small. There is reason to believe, how-

ever, that the specific gravity, and thus also the salt content, varies periodically at one and the same spot in the course of the 24 hours. When we consider, for instance, the elongated, narrow shape of this lake, which has no outlet, and the great difference in degree of evaporation between day and night, which has more or less the same effect as a variation in the quantity of water introduced in the course of the 24 hours, it may well be imagined that these conditions might give rise to a corresponding periodicity in the current movements, and in the level of the surface water. This must make itself apparent in variations of the specific gravity at the same place in the lake periodically throughout the 24 hours, and may also explain the difference noted between samples taken at the same depth but at different times. That the samples were procured from many different stations also furnishes occasion for difference; but in reality, samples taken at different times of the day, but at the same depth, and near the surface, seem to show that the specific gravity increases towards noon, when evaporation is at its height, decreasing gradually thereafter throughout the afternoon. As the samples from the same depth were taken on widely different days, and are few in number, these features are merely indicated here, and will not be further illustrated by tables or graphs.

We can also find in the literature statements which in some degree confirm the idea of such variations. Putnam Cady<sup>1)</sup> for instance, observed, during a period of calm, on three successive nights, a rather sudden, flood-like rise of the water, which produced heavy breakers. Measurements of the variation of the surface would have been of very great value, and would presumably have given the above observations a surer and more quantitative character.

b. Variation of the salt content with depth. Rise in the level of the water.

The tables from the different stations show, as above mentioned, very much the same course. The variation of the salt content with the depth, or of the water sample's content of one of the ions give, roughly speaking, the same view. Fig. 9 shows the variation in the silver equivalent with the depth at St. XI. The silver equivalent per 1000 gr. of the water samples is taken

---

<sup>1)</sup> Quarterly Stat. P. E. F. (1901).

as ordinate, the depth in metres being the abscissa. The curve is of the characteristic shape. Down to 3 metres depth the silver equivalent is the same; here, however, a minor rise takes place. Down to this depth, presumably, the action of the waves is effective, giving the whole upper layer the same composition. From 3 metres down to 20 the silver equivalent is about the same, rising then rapidly in the course of 7 metres from 380 to 480. After this, the rise becomes more gradual. At 50 metres depth, the equivalent value is 540. We have no samples from St. XI from depths beyond 50 m, but from other stations samples have been taken down to 300 metres. From these, we have the following values for the silver equivalent: 60 metres 550, 100 metres 553, and 300 metres 554.6.

The curve suggests that there are, so to speak, two distinct strata of liquid in the lake, the boundary between them being extraordinarily sharply defined. One might imagine, that the high degree of evaporation led to the formation of crystals at the surface; these, sinking to the bottom, would at first, while the difference between their specific gravity and that of the water was great, sink rapidly, and therefore be only slightly dissolved, whereas in the lower water layers, they would sink more slowly, and here dissolve. No crystals have, however, been observed on the surface of the water; and moreover, a crystal would probably be dissolved before sinking down to the 20 metre limit where the boundary lies. Again, the break at 20 metres depth cannot be taken as the lower limit of wave action, as it is too deep down for this.

The best mode of explaining these features seems to me to suppose that evaporation during recent times — the past hundred years — has, for some reason or other, been unable to keep pace with the inflow of water. This would produce a rise in the water level, and the water in the new layers above would be of slighter salinity.

It will be seen then, that Fig. 9 give rise to the same observations as does the study of older analyses of water samples from the Dead Sea. But also direct observations in the course of years bear witness to such a rise of water level. Blankenhorn<sup>1)</sup> for instance, states that a path leading along the shore

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<sup>1)</sup> Naturwissenschaftliche Studien am Toten Meer und im Jordantal. Berlin (1912).

of the lake from Wadi-el-Modschub to Wadi-el-Guvenach, used in 1807, was partly under water in 1874. Near this path there

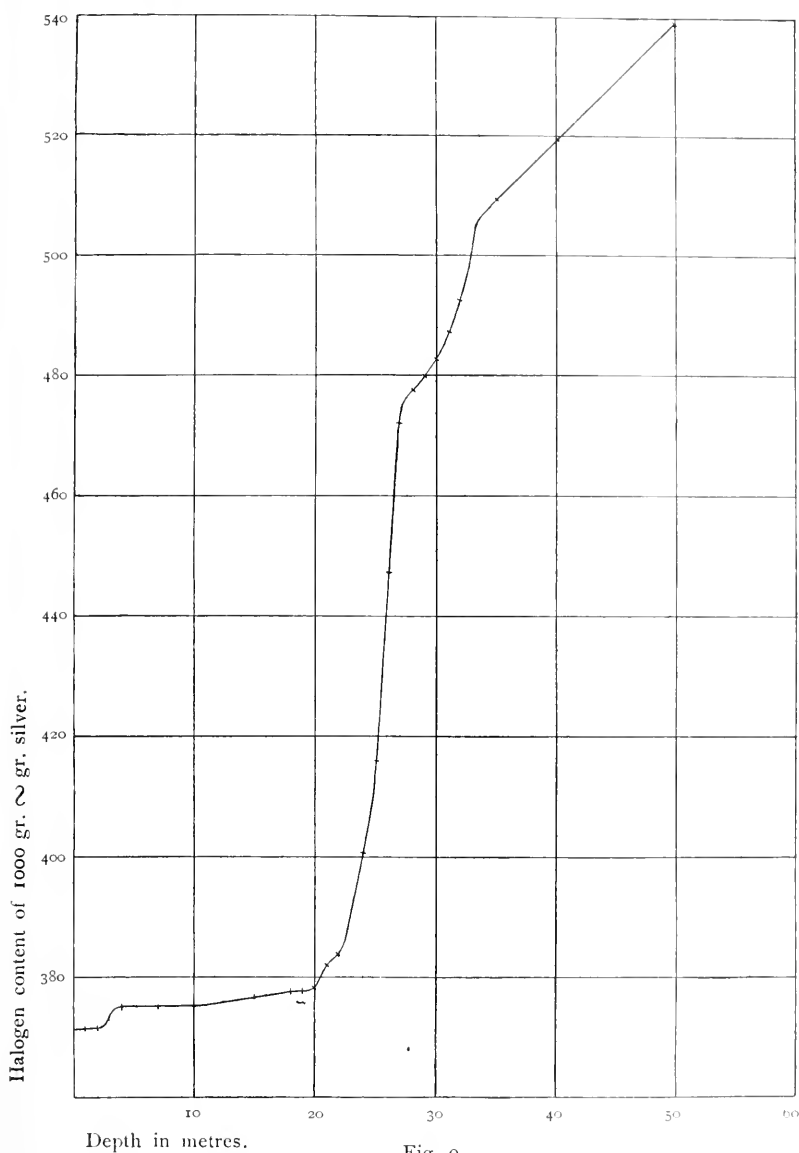


Fig. 9.

was an oil well, which now lies entirely beneath the surface of the lake. Several other passages in Blanckenhorn contain observations as to the rise of the water level; in one place, for

instance, he describes how, standing on an eminence with a view out over the lake, one could follow the line of the valleys far out into the water. In another place he gives a picture of a group of dead tamarisks standing out in lake. There is also, on an old English chart, a small island marked in the north end of the lake, which island was seen as late as 1864 by Louis Lartet, but has since disappeared. This suggests a comparatively rapid rise in the level of the lake.

Blanckenhorn suggests that the water level must have risen between 1.5 and 2 metres in the course of 6 years (p. 91). If a gradual rise had taken place throughout a hundred years, this would correspond to a rise of between 25 and 33 metres, i. e. just in the area where the great break in specific gravity is found.

As to the cause of this rapid alteration in the state of the lake it is not easy to say anything with certainty.

It must be supposed that the evaporation and inflow of water about the year 1800 were more or less equal, for the water at the surface must then have been nearly saturated with salt, and the lake has not, as we know, been dried up at any time within the range of history. If a change of climate, or increase in the inflow of water, should have occasioned the rise, then these changes must have been of a curiously violent and sudden character; and we should expect to find some mention in the literature of such changes taking place; but we have nowhere found any description of such changes. The following observations might perhaps suggest that another cause than that of alteration in the climate, or in the quantity of water introduced, may have led to the rise in the water level of the lake. There are oil wells near the Dead Sea; mention has already been made of one of them. Asphalt is often found drifting in the lake, which undoubtedly points to the presence of an oil well. It might, then, be imagined that 100 years ago, a layer of oil spread over the surface of the lake, reducing the evaporation to such a degree that the inflow of water exceeded the loss by evaporation. Unanimous descriptions furnished by travellers of the streaks of foam or froth dividing up the lake into different coloured fields (blue and green) might in reality suggest the possibility that there was a layer of oil on the surface.

The explanation may perhaps at first appear somewhat fantastic; it has one advantage however, in that it explains, in a



simple manner, the rapid and regular rise which has taken place during the past hundred years.

### c. $\text{SO}_4$ determinations.

The determinations of  $\text{SO}_4$  show, for all stations, the same course, characterised in graphical form in the case of Station XI (Fig. 10). The ordinate is equivalents of  $\text{SO}_4$  per litre, the abscissa depth in metres. The marked decline in the  $\text{SO}_4$  content corresponds exactly to the marked increase in the salt content; in this connection specially in the calcium content. The product of  $\text{SO}_4$  equivalents and calcium per litre is, as shown in Table 16, constant:

TABLE 16.

Depth in metres	a	b	$a \times b$
	equiv. $\text{SO}_4$	equiv. Ca	
2	0.0175	0.523	$9.2 \times 10^{-3}$
10	0.0178	0.525	9.3 »
20	0.0181	0.529	9.6 »
25	0.0170	0.594	10.1 »
30	0.0148	0.713	10.6 »
35	0.0128	0.762	9.8 »
40	0.0121	0.782	9.5 »
50	0.0114	0.817	9.3 »

We must then suppose that the water is saturated with  $\text{CaSO}_4$ , that is to say, it precipitates gypsum. The bottom samples from the Expedition also contained large quantities of this.

### d. Determinations of Oxygen and Hydrogen Sulphide.

The determinations of oxygen and hydrogen sulphide show that a relationship exists between the oxygen content and that of hydrogen sulphide. This is here shown as regards Station XI, in Fig. 11. The determinations of oxygen are indicated by black circles, those of hydrogen sulphide with white. The ordinates indicate cc ( $0^\circ$ , 760 mm) per litre. The abscissa is the depth in metres. It seems as if an oxidation of the hydrogen sulphide must taken place in the upper water layers. The upper branch of the hydrogen sulphide curve and the oxygen curve show a course which fits in very well in qualitative respects. There can hardly be any doubt that the hydrogen sulphide curve would have extended its rising branch (a) if there had not been oxygen present.

The content of oxygen in the water shows, at the surface, an extremely high value, viz. 3.56 cc oxygen ( $0^{\circ}$ , 760 mm) per litre. The water is in reality saturated, or almost saturated, with oxygen. From Station I we have a sample taken just at the surface; the normality in respect to halogen was 1.6. In Landolt Bornstein's Tables (1912) we find, p. 604, a Table showing

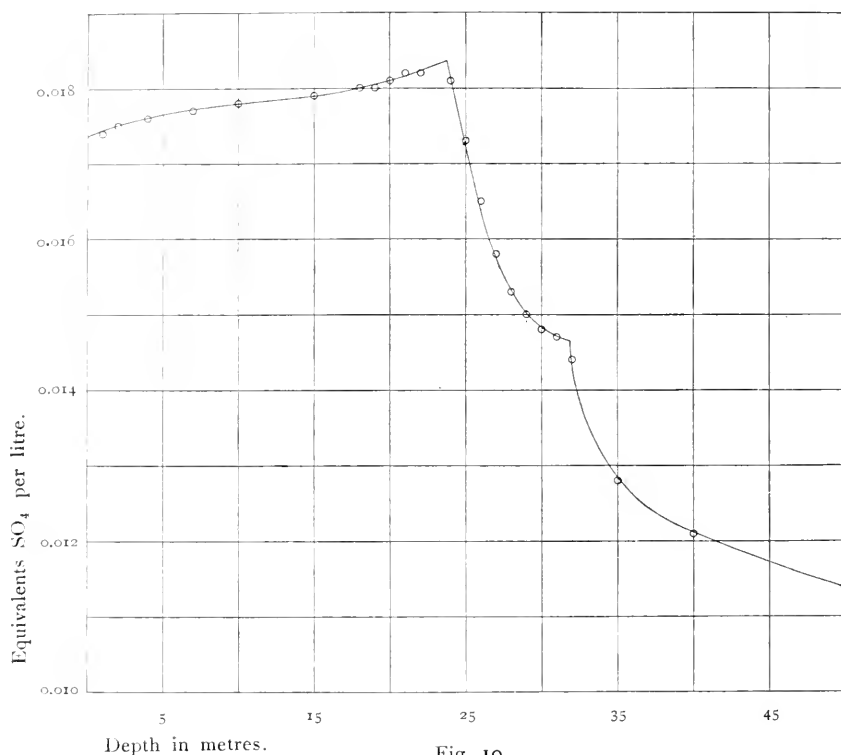


Fig. 10.

the solubility of oxygen in sodium chloride solutions, from which the following figures are quoted:

Temperature  $25^{\circ}$ , partial pressure 760 mm, 1 litre 1 n NaCl absorbs  
 20.4 cc oxygen ( $0^{\circ}$ , 760 mm).  
 " " " " 760 mm, 1 litre 2 n NaCl absorbs  
 14.5 cc oxygen ( $0^{\circ}$ , 760 mm).

From this, by interpolation, we obtain, for 1.6 n NaCl solution, 16.9 cc oxygen ( $0^{\circ}$ , 760 mm). As the partial pressure of oxygen in the atmosphere is abt.  $\frac{1}{5}$  of 760 mm, a sodium chloride solution

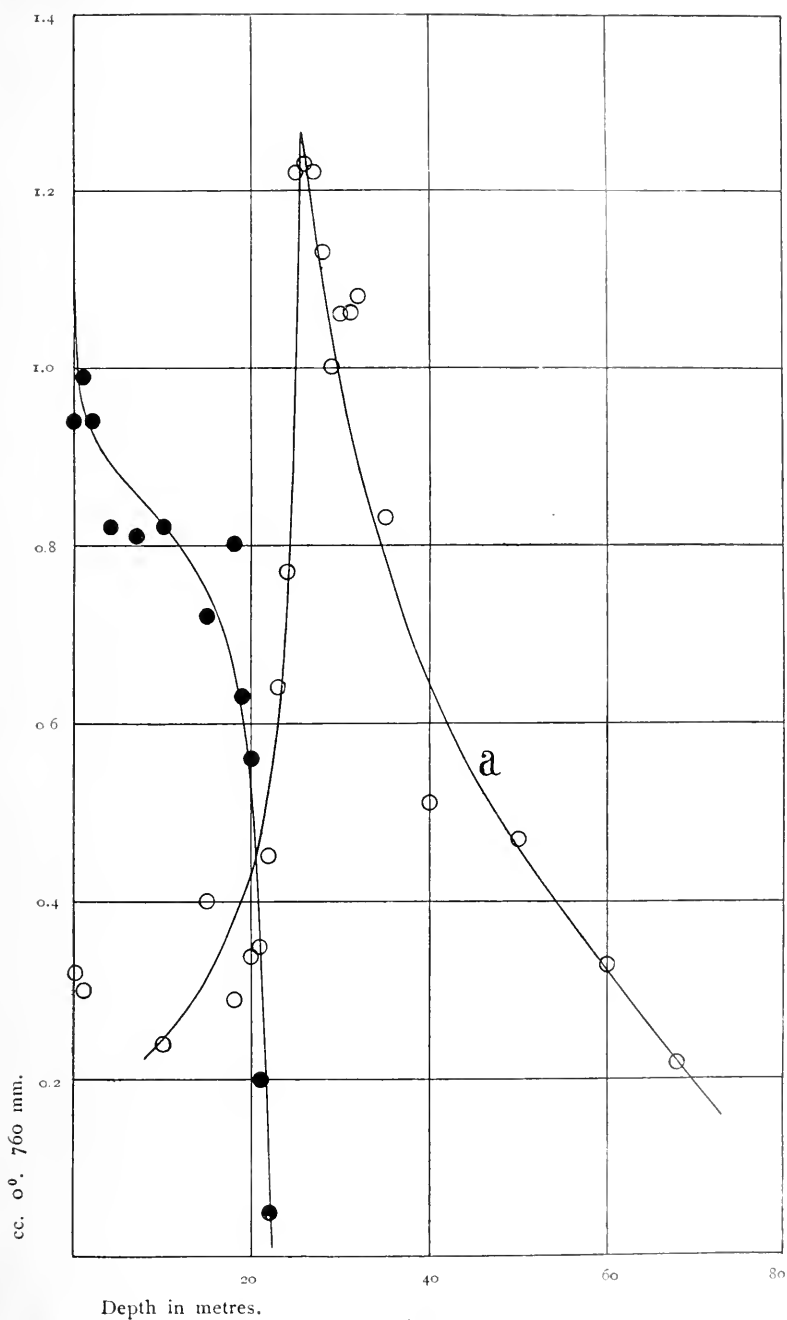


Fig. 11.

of this sort could at the utmost contain 3.4 cc oxygen ( $0^{\circ}$ , 760 mm). This figure agrees very well with the value found for the oxygen content at the surface of the Dead Sea.

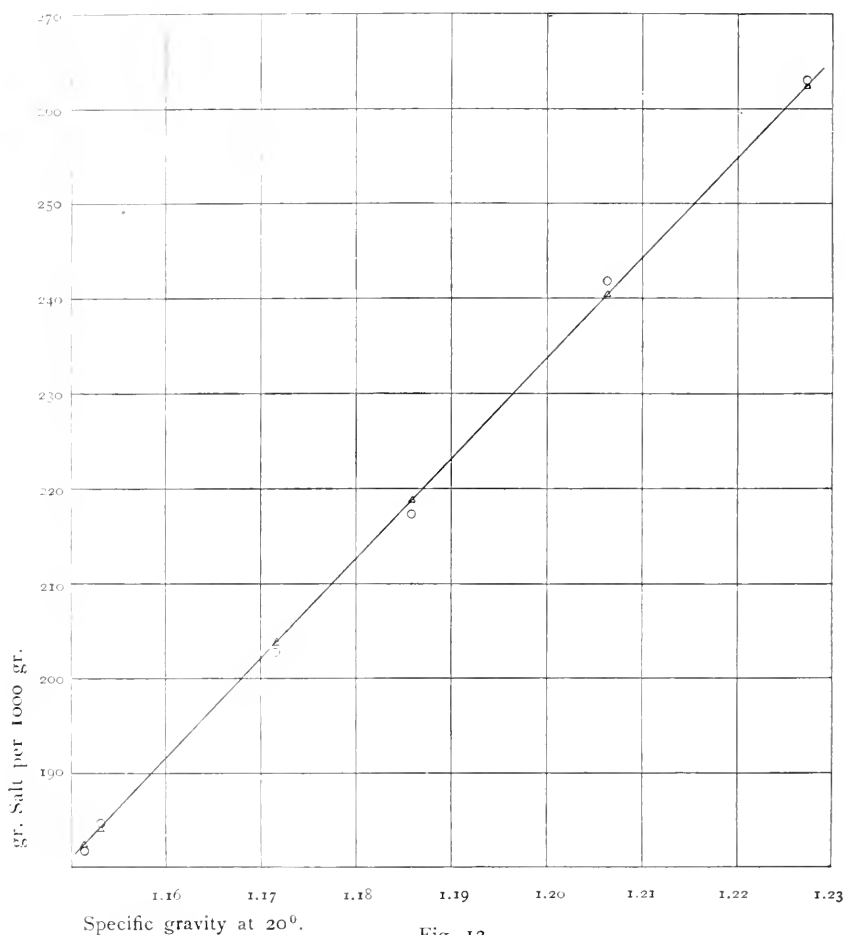


Fig. 12.

#### e. The Bottom Samples.

Among the material from the Expedition was a single bottom sample, taken at a depth of 83 metres off Wadi Ghuwer. The samples contained, in addition to great amounts of ooze, abundance of calcium carbonate, some gypsum, as already noted, and a few well developed crystals of sodium chloride. That there must be some formation of sodium chloride is evident from the figures in the Tables in Section III, as the quantity of sodium

decreases in proportion, so that we have, for St. XI, the ratio between equivalents of sodium per litre and sum of negative equivalents per litre at 1 m depth, amounting to 0.294, at 30 metres 0.274 and at 50 metres 0.266. The quantities of the other ions correspond. This fits in entirely with what we should find if the sodium chloride crystallised out. Only traces of carbonate can be found in the water samples from the Dead Sea, despite the fact that the springs and rivers furnishing water to the lake,

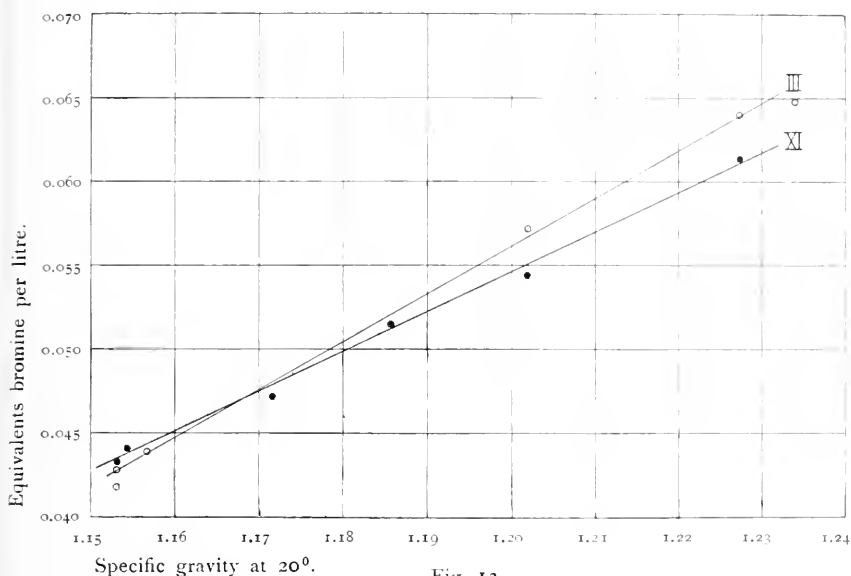


Fig. 13.

Jordan included, are extremely rich in carbonates. The high content of calcium ions in the Dead Sea is probably the cause of this.

#### f. Salt determination.

The salt determinations which have been made refer to Station XI; the method employed is described in Chapter II and has yielded extremely good results. Fig. 12 shows the salt content per 1000 gr. (Ordinate) as a function of the specific gravity at 20° (Abcissa). The quantities of salt found are indicated by circles, those calculated from the analyses by triangles. The greatest difference amounts to 0.8 ‰.

#### g. Bromide determinations.

In Fig. 13 the bromide content from Station III (white circles) and Station XI (black circles) is taken as ordinate, the abscissa

being the specific gravity at 20°. It seems as if the quantity of bromide at the southern station (St. III) increases more rapidly with the depth than at the northern station (St. XI).

#### h. Terreil's Analyses.

In the first chapter, one of Terreil's analyses is shown in Table 2<sup>1</sup>). The water sample from which this analysis was made was taken near the little island which was to be found in the northern end of the Dead Sea; i. e. from the same area as Professor Brühl's. The analysis agrees very well with those from our Expedition. Two more of Terreil's analyses, likewise originating from the area whence our samples were procured, give, however, a result differing considerably from ours.

Without wishing to criticise Terreil's analyses further, we feel nevertheless bound to say that his conclusions can hardly be maintained to the full. What is meant by this will best be seen by reproducing, in part, the summary of results put forward by Terreil in his paper:

1. The specific gravity of the water in the Dead Sea increases with the depth.
2. The water from the Dead Sea is not everywhere of the same composition at the same depth. Thus water taken 5 nautical miles east of Wadi Mrabba contains four times more calcium than water from 5 nautical miles east of Ras Feschkah, but the latter contains about twice as much sodium as the former.
3. The water varies considerably with the depth, and at different places in the sea, the salt content differs at one and the same depth. Thus water taken at 60 metres depth east of Wadi Mrabba contains more salt than water at 200 metres depth east of Ras Feschkah.
5. The proportion between the quantities of the different elements contained in the salts from the Dead Sea is at different depths but in the same vertical line, the same.

It will be seen that 1 and 5 coincide entirely with the view afforded by the analyses from the Brühl's expedition, whereas 2 and 3 differ essentially from this. The area of the lake investigated in this paper has shown that the proportion between the quantities of the different ions in the salt mixture is throughout

<sup>1</sup>) Comptes rendus **62**, 1329 (1866).

essentially the same. The area comprises but a relatively small portion of the lake; it may, however, reasonably be considered large enough for us to expect similar conditions to obtain in the greater part of the lake, and at any rate in the neighbouring areas, from which Terreil's analyses are derived. The fact that about half the water supply of the Dead Sea is furnished by the River Jordan seems further to confirm these observations. There is of course, the possibility that the area treated in this paper may derive its supply of salt from the River Jordan to a much higher degree than the remaining portions of the lake; that this area is, so to speak, dominated by the Jordan. Other parts may obtain salts from other sources, and the composition of the salts in solution in the water may therefore well be somewhat different from that in the "Jordan Area".

It would be very interesting to have these points further investigated. The eight analyses given by Terreil from various depths and various places in the lake have to our mind by no means settled the question

*August 1926.*

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# ETUDES SUR LES PROTEINES.

PAR

S. P. L. SORESENSEN.

## X. SUR LE POIDS SPÉCIFIQUE ET LE POUVOIR ROTATOIRE OPTIQUE DES SOLUTIONS D'ALBUMINE.

PAR

HANS JESSEN HANSEN<sup>1</sup>.

Si l'on peut considérer une solution d'une substance proteique comme un système à deux phases miscibles sans subir une modification de volume, la protéine représentant la phase dispersée, alors que le liquide plus ou moins salin qui l'entoure serait le milieu de dispersion, il est évident que le poids spécifique des systèmes ayant le même milieu de dispersion sera une fonction linéaire de la teneur en protéine, autrement dit, de la phase dispersée, et le poids spécifique de la protéine pourra être calculé d'après le poids spécifique du mélange, si l'on connaît la composition de ce dernier et le poids spécifique du milieu de dispersion. Et inversement, si ce calcul donne, pour le poids spécifique de la protéine, des valeurs variant avec la composition, on aura la preuve que le mélange ne s'est pas réalisé sans modification de volume.

Or, quand une solution d'albumine d'œuf cristallisable servant de liquide intérieur est mise en état d'équilibre osmotique avec un liquide extérieur exempt d'albumine<sup>2</sup>, puis qu'on mélange ensemble ces deux liquides, l'on constate que le volume du mélange est égal à la somme des deux volumes mélanges, ainsi que cela ressort des tableaux dressés ci-dessous, dont le 1<sup>er</sup> se

<sup>1</sup>) Conférence faite devant la Réunion des Chimistes Scandinaves, 1920, à Helsingfors; en partie, remaniée.

<sup>2</sup>) La marche à suivre pour réaliser cet état d'équilibre, a été expliquée dans les Comptes-rendus du Laboratoire Carlsberg, XII, pages 290 et suivantes. (1917).

Tableau I.

Préparation s. D. 4.

Poids spécifiques de mélanges d'une liqueur intérieure de poids spécifique 1.06609 (18°) avec 3 g. 787 N d'œuf = 27 g. 077 d'hydrate d'œuf et 0 g. 0058  $\text{NH}_3\cdot\text{N} \sim 0\text{ g. } 0273 \text{ AmSO}_4$  dans 100 g et une liqueur extérieure de poids spécifique 1.000007 (18°).

Nos	Liquueur intérieure		Liquueur extérieure		Poids spécifique		T ÷ C
	g	Cc.	g	Cc.	Trouvé	Calculé	
1	18.8580 <sub>2</sub>	17.6740 <sub>2</sub>	5.0477 <sub>2</sub>	5.0476 <sub>2</sub>	1.05211 <sub>11</sub>	1.05211 <sub>4</sub>	÷ 0.4
2	15.4407 <sub>6</sub>	14.4713 <sub>1</sub>	9.8292 <sub>3</sub>	9.8291 <sub>6</sub>	1.03997 <sub>8</sub>	1.03991 <sub>1</sub>	+ 6.7
3	10.1909 <sub>1</sub>	9.5511 <sub>4</sub>	14.9484 <sub>11</sub>	14.9483 <sub>11</sub>	1.02610 <sub>2</sub>	1.02612 <sub>1</sub>	÷ 1.8
4	5.4524 <sub>1</sub>	5.1105 <sub>11</sub>	19.7138 <sub>2</sub>	19.7136 <sub>11</sub>	1.01376 <sub>2</sub>	1.01379 <sub>5</sub>	÷ 3.3
5	5.2761 <sub>11</sub> (4.1624 <sub>6</sub> )	5.0147 <sub>8</sub> (3.9011 <sub>2</sub> )	20.1522 <sub>1</sub> (21.2659 <sub>7</sub> )	20.1521 <sub>11</sub> (21.2658 <sub>2</sub> )	1.01044 <sub>4</sub>	1.01039 <sub>11</sub>	+ 5.4 <sup>*)</sup>

\*) Pour l'essai N° 5 le mélange N° 1 a été dilué de façon à correspondre à une dilution du liquide original, comme indiqué par les valeurs mises entre parenthèses.

Tableau II.

Poids spécifique de mélanges d'une liqueur intérieure avec 2 g. 5834 de N protéique  $\sim 22\text{ g. } 476$  d'hydrate d'œuf et 2 g. 4291  $\text{NH}_3\cdot\text{N} \sim 11\text{ g. } 457 \text{ Am}_2\text{SO}_4$  dans 100 g (préparation I. d. Æ. 14) et d'une liqueur extérieure contenant 3 g. 1198  $\text{NH}_3\cdot\text{N} \sim 14\text{ g. } 7148 \text{ Am}_2\text{SO}_4$  dans 100 g.

Nos d'essais	Le mélange contient				Poids spécifique du mélange		Différence T÷C	Poids spécifique de l'hydrate d'œuf	
	Liquueur intérieure		Liquueur extérieure		Trouvé	Calculé		Calculé	Moyennes
	g	cc	g	cc					
	∞ 0	∞ 0	0 ∞	0 ∞	1.11584 <sub>5</sub> 1.08561 <sub>9</sub>			1.23697	
1	21.7208	19.4657 <sub>9</sub>	5.5420	5.1049 <sub>3</sub>	1.10968 <sub>4</sub>	1.10956 <sub>5</sub>	+ 11.9	1.23780	1.23742
2	22.1753	19.8731 <sub>11</sub>	5.5909	5.1499 <sub>7</sub>	1.10964 <sub>1</sub>	1.10962 <sub>4</sub>	+ 1.7	1.23709	
3	22.0235	19.7370 <sub>11</sub>	5.4039	4.9777 <sub>1</sub>	1.10981 <sub>5</sub>	1.10975 <sub>7</sub>	+ 5.8	1.23737	
4	16.5540	14.8354 <sub>11</sub>	10.8420	9.9869 <sub>3</sub>	1.10375 <sub>7</sub>	1.10368 <sub>4</sub>	+ 7.3	1.23767	1.23769
5	16.6460	14.9178 <sub>5</sub>	10.8353	9.9807 <sub>6</sub>	1.10377 <sub>8</sub>	1.10372 <sub>6</sub>	+ 5.0	1.23740	
6	16.5140	14.7995 <sub>5</sub>	10.7406	9.8935 <sub>3</sub>	1.10384 <sub>2</sub>	1.10373 <sub>4</sub>	+ 10.8	1.23799	
7	11.0765	9.9265 <sub>11</sub>	16.2011	14.9233 <sub>1</sub>	1.09778 <sub>1</sub>	1.09769 <sub>3</sub>	+ 8.8	1.23822	1.23737
8	10.9733	9.8340 <sub>7</sub>	16.4233	15.1280 <sub>6</sub>	1.09751 <sub>4</sub>	1.09752 <sub>7</sub>	÷ 1.3	1.23679	
9	10.9051	9.7729 <sub>6</sub>	16.2759	14.9922 <sub>1</sub>	1.09755 <sub>6</sub>	1.09754 <sub>6</sub>	+ 1.0	1.23710	
10	5.5774	4.9983 <sub>7</sub>	21.4118	19.7231 <sub>3</sub>	1.09176 <sub>7</sub>	1.09173 <sub>11</sub>	+ 3.7	1.23802	1.23770
11	5.5915	5.0110 <sub>11</sub>	21.9482	20.2172 <sub>2</sub>	1.09165 <sub>1</sub>	1.09162 <sub>3</sub>	+ 2.8	1.23780	
12	5.5467	4.9708 <sub>5</sub>	21.5875	19.8849 <sub>7</sub>	1.09167 <sub>4</sub>	1.09166 <sub>4</sub>	+ 1.0	1.23728	

rapporte à une solution pratiquement dépourvue de sel, tandis que le II s'applique à une solution contenant du sulfate d'ammonium et, en somme, si riche en sels d'ammonium, qu'elle se rapproche de la limite de précipitation.

Du poids spécifique trouve à celui calculé dans la supposition que le volume du mélange soit égal à la somme de ses parties, la différence, on le voit, est tellement minime qu'elle avoisine la limite des erreurs d'expériences, d'où il semble légitime de conclure que la supposition énoncée au début de ce mémoire est bien juste, en tout cas avec une grande approximation.

Dès lors, nous allons appliquer le précédent raisonnement à des solutions d'ovalbumine cristallisable (hydrate d'œuf) à teneur plus ou moins élevée en sulfate d'ammonium. Les éléments sur lesquels portent et sont fondés nos considérations et nos calculs, sont ceux-ci :

1° Teneur en N protéique dans 100 grammes du mélange

2° — - N ammoniacal - - - - -

3° Poids spécifique du mélange

4° — - - milieu de dispersion

5° Facteur d'hydrate d'œuf, c'est-à-dire le facteur par lequel il faut multiplier le N protéique pour obtenir le poids de la protéine hydratée dissoute.

Les trois premiers de ces facteurs seront établis par des essais. Les deux derniers ont été déjà déterminés par des essais antérieurs et se trouvent indiqués dans des tableaux<sup>1)</sup>. L'emploi de ces derniers présente cependant un caractère quelque peu précaire, en ce sens que, entre la composition du milieu de dispersion et le facteur d'hydrate d'œuf, il existe une dépendance réciproque qui, à la rigueur, exige encore une détermination (par exemple, de la teneur en ammoniacale d'une solution exempte d'albumine et se trouvant en équilibre osmotique avec la mélange expérimenté). Comme, cependant, les variations auxquelles est sujet le facteur en question sont relativement minimes, on pourra — en tout cas en vue d'une première approximation — déterminer ce facteur par un simple calcul estimatif.

Voici, à titre d'exemple, quelques calculs faits de la manière que nous venons d'indiquer :

<sup>1)</sup> Comptes-rendus du Laboratoire Carlsberg, XII, 158, 364. (1917).

N d'œuf du mélange dans 100 g = 4 g.2127

N de  $\text{NH}_3$  - — — 100 - = 0 g.7866

Poids spécifique du mélange = 1.09867.

Le volume de 100 g de mélange sera donc de 91<sup>cc</sup>.0192.

Il en résulte que le mélange contient, par 100 g, 3 g.71 d' $\text{Am}_2\text{SO}_4$ , 3 g.7 de sulfate dans 100 g demandent le facteur d'hydrate d'œuf 8.4<sup>1)</sup>, ce qui aurait pour conséquence que 100 g de solution renfermeraient 4 g.2127 · 8.4 = 35 g.30 d'hydrate d'œuf, résultat qui à son tour donne 64 g.61 de milieu de dispersion, dont 3 g.71 d' $\text{Am}_2\text{SO}_4$ , le reste: 60 g.90 étant de l'eau. Par conséquent, le milieu de dispersion contiendrait, au moins,  $\frac{3.71 \cdot 100}{60.9}$  = 6 g.1  $\text{Am}_2\text{SO}_4$  environ par 100 g d'eau. Le facteur y correspondant est 8.7, et nous trouvons ainsi par 100 g de solution 4 g.2127 · 8.7 = 36 g.65 hydrate d'œuf et 63 g.35 de milieu de dispersion (savoir 59 g.64 d'eau et 3 g.71 d' $\text{Am}_2\text{SO}_4$ ). Le milieu de dispersion contient donc maintenant, au moins, 6 g.22 de sulfate d'ammonium par 100 g d'eau. En cherchant le facteur y correspondant, on trouvera qu'il ne diffère pas de celui que nous avons admis: 8.7. Dès lors, nous pourrions nous arrêter à cette valeur et, conséquemment — vu qu'une dissolution de 6 g.22  $\text{Am}_2\text{SO}_4$  dans 100 g d'eau a un poids spécifique de 1.03297<sup>2)</sup> —, nous constatons que

le volume de 100 g de mélange	est de 91 <sup>cc</sup> .0192
- — du milieu de dispersion	- - 61 <sup>cc</sup> .3263
- — de l'hydrate d'œuf	- - 29 <sup>cc</sup> .6929
le poids spécifique de l'hydrate d'œuf	- - 1.23431.

En procédant de cette façon, on a effectué plusieurs séries d'essais, en faisant varier les trois facteurs suivants:

- la concentration en hydrate d'œuf ou N protéique
- - sulfate d'ammonium et
- - ions hydrogène.

Les résultats de quelques-uns de ces essais sont réunis dans les tableaux III et IV.

Le tableau III se rapporte à des mélanges d'une solution de protéine concentrée et dialysée (à très peu près, exempte de sul-

<sup>1</sup> Comptes-rendus du Laboratoire Carlsberg, XII, 368.

<sup>2</sup> *Ibidem*, 158.

**Tableau III.**

Poids spécifique de l'hydrate d'œuf en solution.  
(Préparation D. A. 9).

Sulfate d'ammo- nium dans 100g	g de N protéique dans 100 g				
	5.26	4.28	3.26	2.22	1.13
0.00	1.30006	1.30011	1.30031	1.30076	1.30090
0.02		1.300	1.300	1.241	
0.06			1.302		
0.15		1.304	1.308	1.313	1.325
0.92				1.314	1.303
1.5		1.266		1.277	1.304
3.7		1.234			
8.2		1.233	1.239	1.288	1.330
15.0			1.233	1.234	1.247
			1.296	1.297	1.316

fate) avec différentes proportions d'eau, ou d'une solution de sulfate d'ammonium de concentration connue. Ce tableau montre en effet que, calculé de cette manière, le poids spécifique de la protéine dissoute augmente en raison inverse de la dilution, lorsque le milieu de dispersion reste inchangé; il est vrai que, surtout dans la dissolution dans l'eau seule, cette augmentation est bien minime. Ce résultat concorde avec les tableaux I et II, en ce sens que les différences y indiquées entre les poids spécifiques calculés et ceux trouvés, même quand elles touchent à la limite des erreurs expérimentales, ont presque toutes le même signe, et justement celui qui indique qu'il s'est produit dans les mélanges une légère diminution de volume. Toutefois, les écarts sont tellement insignifiants, que l'on peut, avec une grande approximation, admettre que l'hydrate d'œuf en dissolution aqueuse a le poids spécifique de 1.300.

De plus, il appert par le tableau que, lorsque la concentration en sulfate augmente, le poids de l'hydrate d'œuf diminue au point d'atteindre, à peu près 1.240 au voisinage de la limite de précipitation. Ceci s'accorde certes bien avec ce fait, que la teneur en eau de l'albumine s'accroît avec la concentration en sel de la solution, mais il faut se rappeler que cette teneur augmentée en eau a été employée dans le calcul et, par conséquent,

Tableau IV.

Influence de la concentration et ions hydrogène sur le poids spécifique de l'hydrate d'œuf dissous.

(Préparations I. d. Æ. 12, D. Æ. 10 et D. Æ. 13).

N protéique dans 100 cc		3.79	3.77	1.86	1.82
Am <sub>2</sub> SO <sub>4</sub> dans 100 cc		0.05	0.28	10.0	15.2
PH	Vf.	4.1	4.2	4.2	
		1.310	1.308	1.262	
		4.1	4.4	4.3	4.4
	—	1.308	1.304	1.249	1.284
		4.4	4.7	4.5	4.6
	—	1.306	1.301	1.249	1.245
		4.7		4.7	4.7
	—	1.304		1.246	1.244
			4.8	4.8	4.9
	—		1.302	1.244	1.243
		5.1		5.1	5.1
	—	1.303		1.242	1.240
		5.4	5.3		5.9
	—	1.311	1.305		1.237

on n'obtient pas par cette voie une preuve de cette teneur en eau. Les valeurs indiquées dans la dernière ligne horizontale du tableau III, pour des poids spécifiques, sont celles qu'on obtiendrait pour l'avant-dernière en employant le même facteur que celui admis pour l'eau seule servant de dissolvant, et l'on voit que ces valeurs ne diffèrent pas notablement de celles de la première ligne pour l'eau seule comme dissolvant.

Cependant, les déterminations consignées dans le tableau II permettent de calculer le poids spécifique de l'albumine dissolue sans qu'il soit nécessaire de connaître le facteur d'hydrate d'œuf. Supposons que la solution saline d'albumen ayant le poids spécifique  $ps_B$  contienne par 100 g b g de sulfate d'ammonium, et que la solution de sulfate d'ammonium étant en équilibre osmotique avec celle-là contienne c g de sel par 100 g, le poids spécifique de cette dernière solution étant désigné par  $ps_D$ , on trouvera alors que 100 g de solution protéique renferment

$$\frac{100}{c} b \text{ g de milieu de dispersion et}$$



$$\frac{100}{c} (c-b) \text{ g de matière protéique;}$$

le volume pouvant être exprimé par

$$\frac{100}{\text{ps}_B} \text{ cc,}$$

comprenant comme volume du milieu de dispersion

$$\frac{100 b}{c \text{ ps}_D} \text{ cc,}$$

d'où il s'ensuit que le volume de l'hydrate d'œuf sera:

$$\frac{100}{\text{ps}_B} - \frac{100 b}{c \text{ ps}_D} = \frac{100 (c \text{ ps}_D - b \text{ ps}_B)}{c \text{ ps}_D \text{ ps}_B} \text{ cc,}$$

donc le poids spécifique de l'hydrate d'œuf:

$$\text{ps}_{\text{alb.}} = \frac{\frac{100}{c} (c-b)}{\frac{100 (c \text{ ps}_D - b \text{ ps}_B)}{c \text{ ps}_D \text{ ps}_B}} = \frac{(c-b) \text{ ps}_B \text{ ps}_D}{c \text{ ps}_D - b \text{ ps}_B}$$

Les valeurs calculées d'après cette formule du poids spécifique de l'hydrate d'œuf, se trouvent consignées dans les deux dernières colonnes du tableau II. On voit qu'elles concordent fort bien avec celles données dans le tableau III au moyen du facteur d'hydrate d'œuf, de sorte que nous avons bien obtenu un appui de l'exactitude de ce facteur<sup>1)</sup>.

Le tableau IV se rapporte à quatre séries d'essais effectuées à des concentrations variées en ions hydrogène, mais par ailleurs avec une composition aussi identique que possible. Les résultats font voir que le poids spécifique de l'hydrate d'œuf diminue très légèrement avec la diminution de la concentration ionique ou avec l'augmentation du  $\text{p}_H$ , et cela aussi bien dans les solutions pauvres en sels que dans celles qui en contiennent beaucoup.

Pour ce qui regarde les solutions riches en sels, on peut se

<sup>1)</sup> Pour ce qui concerne le fait que le facteur d'hydrate d'œuf,  $F$ , s'accroît en même temps que la teneur en sels du milieu de dispersion, il y a lieu de faire observer que cela ne signifie pas seulement que la teneur en eau de l'hydrate d'œuf augmente, mais encore qu'avec la teneur saline du milieu de dispersion s'accroît aussi la quantité de sels entrant dans la phase dispersée constituée par l'hydrate d'œuf\*), de sorte que l'augmentation subie par le poids des particules d'hydrate d'œuf consécutivement à l'accroissement de la facteur  $F$  n'est pas due uniquement à une absorption d'eau mais aussi à une incorporation des autres constituants du milieu de dispersion.

\*) Voir les Comptes-rendus du Lab. Carlsberg, XII, 39 et suiv., 357 et suiv.

demander si le poids spécifique de la solution exempte d'albumine change avec le  $p_H$ . Or, des essais ont montré qu'un mouvement analogue semble avoir lieu ici encore; toutefois, il ne dépasse jamais les limites des erreurs expérimentales.

Avant de quitter ce sujet, nous tenons à ajouter que les anomalies qu'on peut relever dans l'allure générale des tableaux III et IV, sont certainement en partie trop grandes pour pouvoir être imputées à des erreurs qu'on aurait faites dans la détermination du poids spécifique; elles doivent provenir d'autres causes, que nous n'avons pas encore pu découvrir.

Au cours de nos recherches, nous avons tâché de déterminer aussi une autre constante de l'ovalbumine cristallisable; nous voulons parler du pouvoir de rotation qu'elle possède vis-à-vis du plan de la lumière polarisée.

Cette constante a été déterminée antérieurement par plusieurs chercheurs; c'est ainsi que F. G. Hopkins<sup>1)</sup> a trouvé  $(\alpha)_D = \div 30^0.7$ , Thomas B. Osborne  $\div 28^0.6 - 29^0.6^2)$ ,  $\div 29^0.13 - 30^0.48^3)$ , Edith G. Willcock<sup>4)</sup>:  $\div 30^0.3 - 31^0.5$ . Les valeurs trouvées par E. G. Young<sup>5)</sup> sont comprises entre les mêmes limites.

D'anciens expérimentateurs — qui ont peut-être opéré sur des matières moins bien définies — avaient trouvé des valeurs s'écartant sensiblement de celles-là.

Nous avons déterminé le pouvoir rotatoire pour les lignes C, D, E et en partie F, au moyen de la lumière spectroscopiquement purifiée d'une lampe de Nernst; plus tard, celle-ci s'étant défaite et ne pouvant pas être remplacée, pour la ligne jaune de mercure,  $\lambda = 579$ . Nous avons constaté que le pouvoir rotatoire est influencé par la concentration des solutions étudiées (de protéine aussi bien que de sel, c. à. d. sulfate d'ammonium) ainsi que par la concentration en ions hydrogène.

Le pouvoir de rotation spécifique de la protéine  $[\alpha]$  fut calculé sur le contenu, évalué d'après Kjeldahl, en azote protéique, à l'aide de la formule

$$[\alpha] = \frac{100 \alpha}{6.4 \cdot N \cdot l},$$

<sup>1)</sup> Journal of Physiology, **25**, p. 306 (1900).

<sup>2)</sup> Journal of the Am. Chem. Soc., **XXI**, p. 482 (1899).

<sup>3)</sup> *Ibid.*, **XXII**, p. 422 (1900).

<sup>4)</sup> Journal of Physiology, **37**, p. 27 (1908).

<sup>5)</sup> Proc. Royal Soc. London, **B. 93**, p. 15 (1922).

Tableau V.

Influence de la concentration saline sur la rotation (Préparation «S»).

100 cc contiennent		pH de la solution	$\alpha_C$	$\alpha_D$	$[\alpha]_{57.9}$	$\alpha_E$	$[\alpha]_F$
g de N protéique	g $\text{NH}_3\text{-N}$						
0.7056	0.695	4.78	23.14	30.59		37.37	53.37
0.7098	0.699	4.80			32.50		
0.7082	0.698	4.80			32.34		
0.7051	1.589				32.24		
0.7190	1.612	4.80			32.50		
0.7328	2.549	4.79	23.03	30.28		37.42	51.97
0.7087	2.492	4.78			32.63		
0.7115	2.522	4.78			32.17		
0.7065	4.320	4.81					
0.7152	4.315	4.70			31.68		
0.7190	4.373	4.79			31.09		

\*) Très incertain.

ou  $\alpha$  représente la rotation observée dans un tube de 1 décimètre de longueur, dans une solution renfermant N g d'azote protéique par 100 cc.

L'influence exercée par la concentration en sel est relativement faible. Elle ressort du tableau V et de la figure 1 y appartenant.

L'influence qu'exerce la concentration des protéines est sensiblement plus grande, ainsi que le montrent les tableaux VI et VII avec les figures 2 et 3.

Dans le tableau VI est consignée, comme on peut le voir, une série d'essais répétés trois fois et dont les moyennes sont reproduites graphiquement dans la fig. 2. Celle-ci montre que la dépendance considérée est très nettement linéaire. Il est vrai que ceci ressort avec moins de netteté des résultats expérimentaux présentés par les figures 1 et 3; mais néanmoins, si l'on examine combien les résultats du tableau VI s'écartent des moyennes, on reconnaîtra que, même pour la dépendance indiquée par les fig. 1 et 3, il est légitime de la qualifier de linéaire, de sorte qu'elle est bien représentée par la ligne pleine. Par contre, la valeur absolue que cette ligne donne pour le pouvoir rotatoire, pourra assurément dans certaines circonstances être entachée de légères erreurs.

L'influence du troisième des facteurs susmentionnés, la concentration en ions hydrogène, avait déjà été observée par Young<sup>1)</sup> et Osborne<sup>2)</sup>. Elle est plus considérable que celle des deux autres concentrations, ainsi qu'il ressort des tableaux VIII à XI et des fig. 4 à 7 y appartenant. Au surplus, ces dernières font

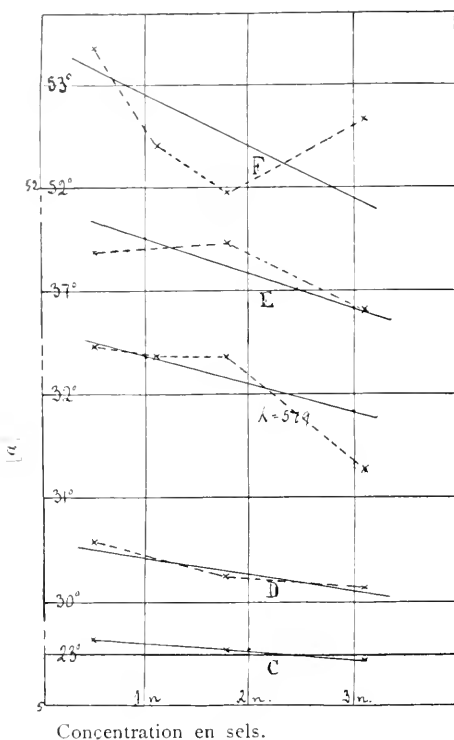


Fig. 1.

voir que cette relation est également linéaire dans les limites des concentrations ioniques considérées, c. à d. au voisinage du point iso-électrique de l'ovalbumine, et, d'autre part, qu'elle n'est pas affectée par la concentration en protéine ni par celle en sel.

Cette action de la concentration en ions hydrogène fournit l'explication de ce double fait constaté par E. Willcock<sup>3)</sup>, que les préparations ayant cristallisé au sein d'un liquide acide possèdent un  $[\alpha]_D$  plus élevé (savoir  $\div 34^{0.5}$ ) et que la recristallisation dans un milieu moins acide avait pour effet de diminuer peu à peu le pouvoir rotatoire de ces préparations.

On sait, en effet, que les solu-

tions acides d'albumine d'œuf donnent naissance à des cristaux renfermant un excès d'acide et que cet excès se réduit peu à peu par recristallisation<sup>4)</sup>.

Quant à l'action exercée par les divers facteurs sur le pouvoir rotatoire vis-à-vis de lumière à largeur d'onde différente, il appert

<sup>1)</sup> Loc. cit.

<sup>2)</sup> Loc. cit. Les déterminations d'Osborne, ayant été effectuées avant que la notion de concentration ionique ait été généralement connue, tendent seulement à établir que le pouvoir rotatoire subit une modification par suite d'une addition de petites doses d'acide ou de base à la solution expérimentée.

<sup>3)</sup> Loc. cit.

<sup>4)</sup> Comptes-rendus du Lab. Carlsberg, XII, 185.

Tableau VI.

Influence exercée sur la rotation par la concentration protéique à  $p_H = 4.86$ ,  $p_{aH} = 4.90$ , et par une concentration saline constante du milieu de dispersion, concentration = 17 g. 253 Am<sub>2</sub> SO<sub>4</sub> dans 100 g d'eau. (Préparation I. d. A.E. 14).

100 cc contiennent		[ $\alpha$ ] <sub>C</sub>	[ $\alpha$ ] <sub>D</sub>	[ $\alpha$ ] <sub>E</sub>	$\alpha$ <sub>F</sub>
g N protéique	g Am <sub>2</sub> SO <sub>4</sub>				
2.8827	12.784	23.04	30.19	36.75	50.02
2.2840	13.449	23.57	30.85	37.63	51.99
2.2894	13.441	23.42	30.44	36.92	51.36
2.3022	13.428	23.48	30.34	37.04	51.21
Moyenne		23.49	30.54	37.13	51.52
1.7230	14.069	23.63	30.65	37.35	51.53
1.7272	14.063	23.38	30.67	37.18	51.16
1.7279	14.064	23.70	30.87	37.62	52.22
Moyenne		23.57	30.73	37.38	51.67
1.1516	14.701	23.68	30.94	37.72	52.10
1.1356	14.718	23.85	31.07	37.84	52.08
1.1376	14.718	23.97	30.91	37.64	52.75
Moyenne		23.83	30.97	37.73	52.31
0.58286	15.330	23.86	31.30	38.06	52.28
0.57259	15.341	23.87	31.06	38.06	52.26
0.57650	15.337	23.58	30.83	37.13	52.85
Moyenne		23.77	31.06	37.75	52.46

Tableau VII.

Influence de la concentration protéique sur la rotation.  
Préparation »<sup>6</sup>/<sub>12</sub>«.

100 cc contiennent		$p_{aH}$	$\alpha_{\lambda = 579}$	
g N protéique	g NH-N		Observé	Corrigé pour $p_{aH} = 4.8$
1.800	1.394	4.75	31 <sup>0</sup> .29	31 <sup>0</sup> .16
1.416	1.375	4.75	31 <sup>0</sup> .41	31 <sup>0</sup> .28
1.312	1.425	4.80	31 <sup>0</sup> .55	31 <sup>0</sup> .55
1.136	1.401	4.81	31 <sup>0</sup> .14	31 <sup>0</sup> .17
0.8634	1.396	4.84	31 <sup>0</sup> .42	31 <sup>0</sup> .52
0.5713	1.405	4.85	31 <sup>0</sup> .67	31 <sup>0</sup> .80
0.2964	1.388	4.89	31 <sup>0</sup> .72	31 <sup>0</sup> .95
0.1509	1.421	4.91	31 <sup>0</sup> .56	31 <sup>0</sup> .85

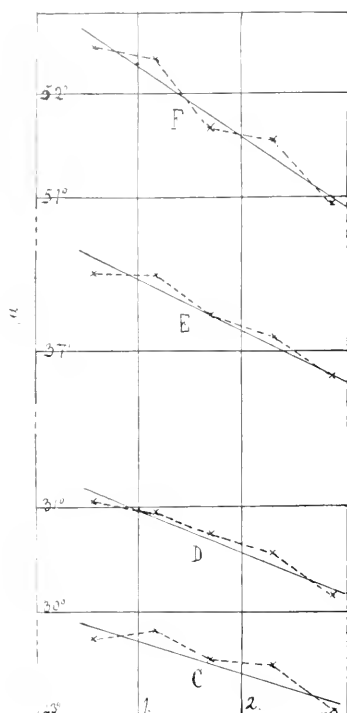
g N protéique dans 100 cm<sup>3</sup>

Fig. 2.

effectuer la détermination dans des conditions identiques, ou bien trouver moyen par des corrections appropriées de rapporter à quelque série de conditions normales les déterminations pratiquées dans des conditions variées.

Or, les matériaux exposés dans ce qui précède, permettent de trouver des corrections tant pour la concentration en sel et protéine que pour celle des ions hydrogène<sup>1)</sup>.

de tous les tableaux et courbes qu'elle augmente lorsque diminue la largeur d'onde de la lumière.

Comme un facteur susceptible d'influer sur le pouvoir rotatoire, il convient de citer encore la température. Le tableau XII indique les rotations observées dans la préparation »S« à des températures variées dans un tube de 400 mm de longueur. »S« contenait 2<sup>s</sup>.574 de N de NH<sub>3</sub> et 2<sup>s</sup>.613 de N d'œuf dans 100 g de solution.  $\rho_{\text{H}} = 4.97$ .

Le tableau montre que dans le domaine expérimenté l'action de la température est tellement faible, qu'il n'est pas nécessaire d'en tenir compte.

Étant donné que, comme nous l'avons vu, la valeur du pouvoir rotatoire de l'albumine d'œuf cristallisable varie selon les conditions expérimentales, il faut, lorsqu'on veut faire la comparaison de différentes préparations d'albumine d'œuf, s'attacher à

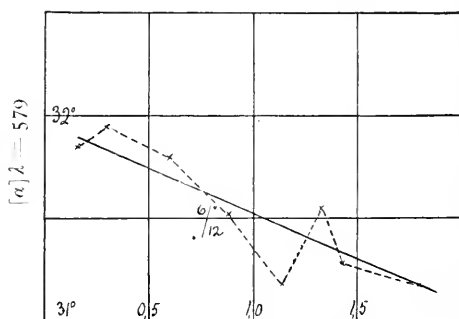
g N protéique dans 100 cm<sup>3</sup>

Fig. 3.

<sup>1)</sup> Le tableau ci-après contient les facteurs de correction dérivés des essais relatés plus haut, facteurs à l'aide desquels et à partir du pouvoir rotatoire

Comme ces matériaux portent sur des préparations diverses d'albumine d'œuf, nous avons, pour être à même de comparer celles-ci entre elles, rapporté moyennant ces corrections toutes nos déterminations à une solution qui, normale sous le rapport du sulfate d'ammonium, contient dans 100 cc. 1 g d'azote d'albumine et présente une activité des ions hydrogène<sup>1)</sup>,  $\text{pH} = 4.80$ . Après avoir de cette façon rapporté aux conditions normales sus-indiquées tous les essais consignés dans les tableaux précédents, on a calculé pour chaque préparation la moyenne des valeurs ainsi obtenues pour le pouvoir de rotation vis-à-vis de chaque ligne de lumière. Ces valeurs sont rassemblées dans le tableau XIII ci-contre. Celles mises entre parenthèses, relatives à la ligne jaune de mercure, sont interpolées entre les autres.

On peut remarquer que les valeurs inscrites sur le tableau XIII pour les différentes substances protéiques, diffèrent quelque peu les unes des autres, bien que, sauf une seule exception, elles sont comprises entre les mêmes limites que celles auxquelles étaient arrivés les chercheurs précédents.

Il se pose maintenant la question de savoir si les écarts constatés sont à regarder comme des erreurs d'expériences au sens propre, c'est-à-dire des erreurs de lecture ou d'analyse, ou bien si elles sont dues à des différences dans les préparations étudiées, qu'il s'agisse de diversités de composition élémentaire ou qu'elles proviennent de ce que la substance considérée n'aurait pas été en équilibre.

Que ce dernier cas puisse se produire bien souvent, on peut le voir par ce fait que, si l'on examine la rotation d'un tube rempli d'une solution de protéine, puis qu'on l'abandonne à la

trouvé à une solution donnée, on est à même de calculer le pouvoir rotatoire pour une modification quelconque de la solution.

	C	D	$\lambda$ 579	E	F
$d[\alpha]/d \text{ sel}$	$\div 0.07$	$\div 0.18$	$\div 0.28$	$\div 0.35$	$\div 0.50$
$d[\alpha]/d N +$	$\div 0.32$	$\div 0.40$	$\div 0.42$	$\div 0.50$	$\div 0.68$
$d[\alpha]/d \text{pH}$	$\div 1.5$	$\div 2.5$	$(\div 2.6)$	$\div 3.0$	$\div 4.4$

Pour la variable indépendante les unités employées sont les suivantes: Pour le sel, les normalités, pour l'albumine, le nombre de g de N par 100 cc.

<sup>1)</sup> Comptes-rendus du Lab. Carlsberg, XV, No 6 (1924); XVI, No 3 (1925).

**Tableau VIII.**

Influence de la concentration en ions hydrogène sur la rotation.  
Préparation I. d. Æ. 12.1.  
100 cc de solution contiennent 1 g.817 N-protéique et 15 g.2 Am<sub>2</sub>SO<sub>4</sub>.

P <sub>a</sub> H	[α] <sub>C</sub>	[α] <sub>D</sub>	[α] <sub>E</sub>	[α] <sub>F</sub>
4.39	24 <sup>0</sup> .42	31 <sup>0</sup> .55	39 <sup>0</sup> .03	53 <sup>0</sup> .65*)
4.59	23 <sup>0</sup> .82	31 <sup>0</sup> .30	38 <sup>0</sup> .61	55 <sup>0</sup> .29*)
4.75	23 <sup>0</sup> .53	30 <sup>0</sup> .80	37 <sup>0</sup> .56	52 <sup>0</sup> .19
4.95	23 <sup>0</sup> .26	29 <sup>0</sup> .71	37 <sup>0</sup> .28	51 <sup>0</sup> .38
5.14	22 <sup>0</sup> .96	29 <sup>0</sup> .76	36 <sup>0</sup> .46	49 <sup>0</sup> .84
5.96	21 <sup>0</sup> .90	28 <sup>0</sup> .86	35 <sup>0</sup> .79	49 <sup>0</sup> .09

\*) Incertain.

**Tableau IX.**

Influence de concentration en ions hydrogène sur la rotation.  
Préparation I. d. Æ. 12.2.  
100cc de solution contiennent 1 g.860 N protéique et 10 g.00 Am<sub>2</sub>SO<sub>4</sub>.

P <sub>a</sub> H	[α] <sub>C</sub>	[α] <sub>D</sub>	[α] <sub>E</sub>	[α] <sub>F</sub>
4.19	24 <sup>0</sup> .96	32 <sup>0</sup> .61	39 <sup>0</sup> .58	54 <sup>0</sup> .50
4.38	24 <sup>0</sup> .69	32 <sup>0</sup> .34	39 <sup>0</sup> .43	54 <sup>0</sup> .47
4.57	24 <sup>0</sup> .40	31 <sup>0</sup> .74	38 <sup>0</sup> .81	53 <sup>0</sup> .68
4.75	24 <sup>0</sup> .05	31 <sup>0</sup> .33	38 <sup>0</sup> .22	53 <sup>0</sup> .02
4.95	23 <sup>0</sup> .62	30 <sup>0</sup> .89	37 <sup>0</sup> .83	51 <sup>0</sup> .94

**Tableau X.**

Influence de concentration en ions hydrogène sur la rotation.  
Préparation D. Æ. 13.  
100 cc de solution contiennent 3 g.795 N protéique et 0 g.0457 Am<sub>2</sub>SO<sub>4</sub>.

P <sub>a</sub> H	[α] <sub>C</sub>	[α] <sub>D</sub>	[α] <sub>E</sub>
4.11	24 <sup>0</sup> .65	32 <sup>0</sup> .25	39 <sup>0</sup> .45
4.17	24 <sup>0</sup> .25	31 <sup>0</sup> .57	38 <sup>0</sup> .71
4.43	24 <sup>0</sup> .03	31 <sup>0</sup> .24	38 <sup>0</sup> .10
4.64	23 <sup>0</sup> .75	30 <sup>0</sup> .66	38 <sup>0</sup> .00
4.68	23 <sup>0</sup> .50	30 <sup>0</sup> .70	37 <sup>0</sup> .50
4.79	22 <sup>0</sup> .87	30 <sup>0</sup> .31	36 <sup>0</sup> .99
4.81	23 <sup>0</sup> .26	30 <sup>0</sup> .32	36 <sup>0</sup> .92
5.09	22 <sup>0</sup> .70	29 <sup>0</sup> .96	36 <sup>0</sup> .67
5.39	22 <sup>0</sup> .77	29 <sup>0</sup> .79	36 <sup>0</sup> .33



**Tableau XI.**

Influence de concentration en ions hydrogène sur la rotation.

Préparation D. Æ. 10.

100 cm<sup>3</sup> de solution contiennent 3 g 763 N protéique et  
0 g 28 Am<sub>2</sub>SO<sub>4</sub>.

$p_{aH}$	$\alpha_D$
4.21	30 <sup>0</sup> .71
4.69	29 <sup>0</sup> .23
4.85	28 <sup>0</sup> .61

**Tableau XII.**

Influence de la température sur la rotation.

Ligne spectrale	Rotation observée 400 mm à		
	11 <sup>0</sup> .5	19 <sup>0</sup> —22 <sup>0</sup>	29 <sup>0</sup>
C	16.85	16.83	16.82
D	22.20	22.11	22.13
E	27.16	27.01	27.01
F	37.51	37.28	37.33

**Tableau XIII.**

Valeurs moyennes de  $[\alpha]$  pour les différentes lignes lumineuses dans les circonstances normales, c. à. d. 1 g N protéique dans 100 cc de solution normale de sulfate d'ammonium,  $p_{aH} = 4.8$ , calculé pour les diverses préparations protéiques, sur la base de tous les essais portés sur les tableaux précédents.

Préparation		$[\alpha]_c$	$[\alpha]_D$	$[\alpha]_{\lambda=579}$	$[\alpha]_E$	$[\alpha]_F$
«S»	Tableau V	22.96	30.33	(31.83)	37.37	52.87
—	—			32.20		
I. d. Æ. 14	— VI	23.99	31.50	(33.00)	38.40	53.27
«6/12»	— VII			31.48		
I. d. Æ. 12.1	— VIII	23.91	31.17	(32.67)	38.81	53.00
— 12	— IX	24.32	31.64	(33.14)	38.69	53.53
D. Æ. 13	— X	24.28	31.39	(32.89)	38.31	
D. Æ. 10	— XI		29.92			

Les valeurs mises entre parenthèses ne sont pas mesurées mais trouvées par interpolation.

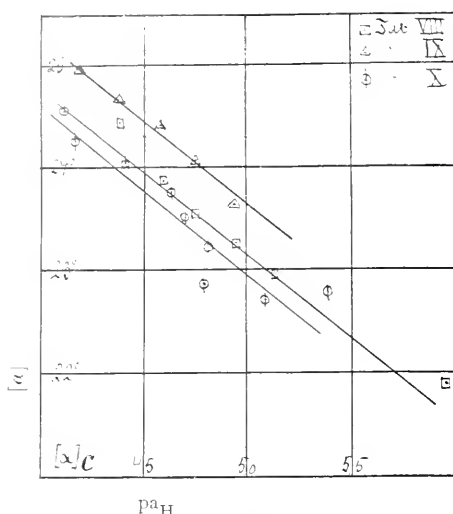


Fig. 4.

décomposition, peut-être de nature bactérienne, subie par la molécule de protéine; il semble bien que l'augmentation de pouvoir rotatoire soit causée par le même phénomène.

Toutefois, il y a aussi possibilité que, nonobstant la purification soignée et les recrystallisations répétées dont elle a été l'objet, l'albumine d'œuf cristallisable soit de composition élémentaire différente; or, que ce fait puisse amener une différence de pouvoir rotatoire, c'est ce que laisse supposer l'essai que nous allons relater<sup>1</sup>.

Une préparation d'albumine d'œuf qui avait été recrystallisée six fois à la manière usuelle dans une solution de sulfate d'ammonium, fut dissoute dans de l'eau, puis dialysée pendant trois semaines env.,

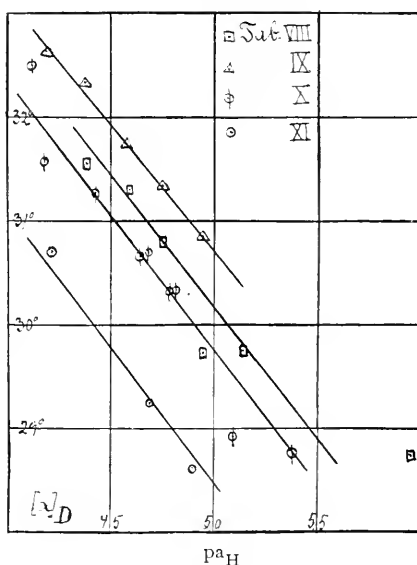


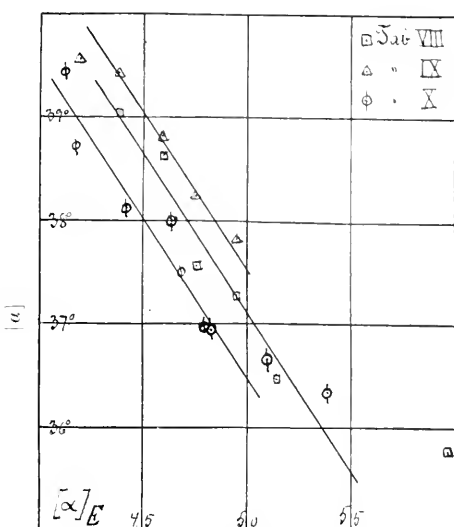
Fig. 5.

<sup>1</sup>) La préparation a été faite par Margrethe Sørensen.

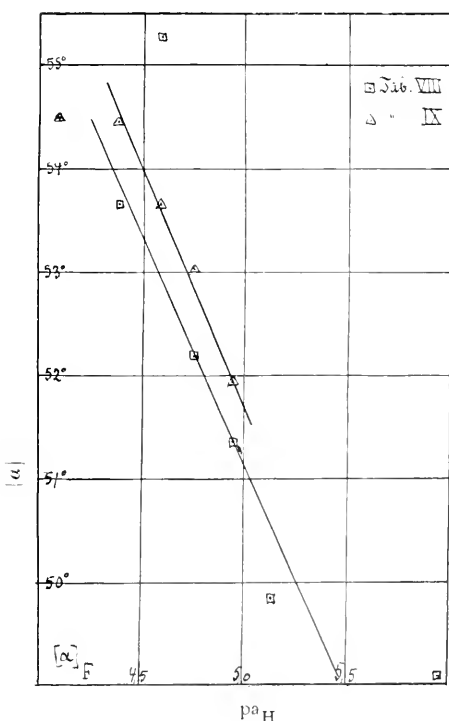
jusqu'à ce qu'elle eût été débarrassée de presque tout le sel y contenu. La proportion entre le phosphore et l'azote de la solution fut établie à  $P/N = 0.00786$ . Ensuite, on la soumit à l'électrodialyse d'après Pauli à travers des membranes de collodion, opération qui eut pour effet qu'une partie de la protéine entra dans le compartiment de l'anode, une autre partie restant dans le vase intérieur de l'appareil.

La substance protéique des deux liquides fut alors amenée à cristalliser à la manière habituelle, et l'on détermina dans chaque fraction la proportion de la teneur en azote à celle en phosphore. Pour les cristaux provenant de la liqueur anodique, la proportion était de  $P/N = 0.00853$ , pour la liqueur intérieure, de 0.00667. On fit dissoudre les cristaux obtenus, et l'on en examina le pouvoir rotatoire.

La solution anodique et dont l'albumine avait traversé une membrane dialytique, présentait la composition suivante: 100 cm<sup>3</sup> contenaient 2 g. 3906 de N protéique et 2 g. 7432 de N de NH<sub>3</sub>;  $p_{aH}$  fut estimé



$p_{aH}$   
Fig. 6.



$p_{aH}$   
Fig. 7.

par mesurage à 4.74, après correction: 4.66<sup>1)</sup>, et montra dans des tubes de 200 mm une rotation de la lumière jaune de Hg de  $\alpha = \div 9^{\circ}.45$ , d'où après application des corrections indiquées plus haut relatives aux circonstances normales (1 g d'albumine par 100 cc d'Am<sub>2</sub>SO<sub>4</sub>,  $\rho_{\text{H}}$  étant = 4.80):

$$[\alpha]_{\lambda}^{\text{Prot}} = \div 31^{\circ}.39.$$

Avec l'albumine provenant de la cellule intérieure et qui n'avait traversé aucune membrane de dialyse, on prépara une solution de la composition suivante:

100 cc contenaient 2 g.101 de N d'albumine et 2 g.6509 de N de NH<sub>3</sub>, le  $\rho_{\text{H}}$  étant estimé par mesurage à 4.79, corrigé à 4.73; cette solution montra dans des tubes de 200 m/m, pour la lumière jaune de mercure, la rotation

$$\alpha = \div 8^{\circ}.496,$$

d'où après correction relative aux circonstances normales:

$$[\alpha]_{\lambda} - 579 = \div 32.07.$$

La différence de pouvoir rotatoire des deux préparations se chiffre par 2<sup>0</sup>/o env., tandis que la précision des estimations analytiques apparaît comme entachée d'une erreur, qui peut s'élever jusqu'à  $\pm 1/2^{\circ}$ /o. Il ressort de là que les divergences observées pour le pouvoir rotatoire se rapprochent de la limite d'erreurs des déterminations, de sorte que, en tout cas jusqu'à plus ample informé, on ne pourra leur attribuer une très grande importance.

*Mars 1927.*

## NOTE ADDITIONNELLE

Après que le présent mémoire avait été mis sous presse, il a paru un travail de F. L. Hewitt<sup>2)</sup> dans lequel il est démontré que chez diverses substances protéiques, et parmi elles l'albumine d'œuf, le pouvoir de rotation optique pour la lumière de largeurs d'onde différentes, peut s'exprimer par l'équation ci-dessous, qui est une forme simplifiée par Lowry<sup>3)</sup> d'une expression plus générale indiquée par Drude.

<sup>1)</sup> Comptes-rendus du Lab. Carlsberg, Vol. 16, N° 3, p. 21, 22.

<sup>2)</sup> Optical rotatory power and dispersion of proteins. The Biochemical Journal, XXI, 216—224. (1927).

<sup>3)</sup> Journal of the Chemical Society, 103, 1067. (1913).

Voici l'équation formulée par Lowry:

$$\frac{1}{a_{\lambda}} = \frac{\lambda^2 - \lambda_0^2}{k}$$

où  $\lambda$  signifie la largeur d'onde de la lumière considérée et  $a_{\lambda}$  le pouvoir rotatoire spécifique qui y correspond, tandis que  $k$  est une constante caractéristique des différents corps, et  $\lambda_0$  la largeur d'onde d'un rayon de lumière absorbé par la substance en question et qui peut de même varier pour les substances particulières.

Il semblait donc être d'un certain intérêt de rechercher si les résultats des investigations dont nous parlons, s'accordent avec ceux auxquels Hewitt est arrivé, et l'on peut alors constater que, si on cherche à calculer un  $k$  et un  $\lambda_0$  à l'aide des séries d'expériences où le pouvoir rotatoire est déterminé pour plusieurs lignes, il est possible dans chaque cas particulier de déterminer ces constantes de telle façon, que les angles de rotation que l'équation de Lowry permet de calculer au moyen d'elles concordent parfaitement avec ce qu'on aura observé. Dans les circonstances normales, on trouvera  $k =$  environ 8.4 et  $\lambda_0^2 =$  0.08 env.

Or, comme  $[a]_{\lambda}$  varie suivant les conditions expérimentales, il s'ensuit évidemment que  $k$  et  $\lambda_0^2$ , ou du moins l'un d'eux, doivent en faire de même. Aussi, en calculant ces constantes pour chacun de nos essais, nous constatons une certaine régularité dans les valeurs obtenues pour  $k$ , et ce de telle sorte que celles-ci augmentent en même temps qu'augmente la concentration en sels (bien que cela ne soit probablement vrai qu'au dedans des limites des erreurs expérimentales), alors qu'au contraire ces valeurs diminuent lorsque la concentration protéique et  $p_{aH}$  augmentent. Les valeurs observées sont comprises entre 7.8 et 9.4. Quant aux valeurs de  $\lambda_0^2$ , par contre, elles oscillent entre 0.056 et 0.090, et l'on ne saurait constater aucune régularité pour ce qui les regarde.

Ainsi donc, en tout cas  $k$ , et peut-être  $\lambda_0^2$  aussi, n'est pas en réalité une constante, mais fonction des conditions expérimentales,  $f(N, c, p_{aH})$ . Nous avons tenté, à l'aide des données obtenues, de calculer ces fonctions comme une somme de termes de correction, mais cette tentative est restée infructueuse. Néanmoins, il ne peut guère y avoir de doute que des recherches de

ce genre ne puissent fournir des résultats précieux en ce qui concerne la définition exacte des substances albumineuses, d'autant que les diverses préparations semblent susceptibles de donner des valeurs différentes pour  $k$  et  $\lambda_0^2$ . Effectivement, en calculant  $k$  et  $\lambda_0^2$  au moyen des valeurs de  $[\alpha]$  établies pour des préparations diverses et réunies dans le Tableau XIII, on trouve que  $k$  varie entre 7.8 et 8.8, et  $\lambda_0^2$  entre 0.07 et 0.08.

Hewitt a encore trouvé un angle de rotation notablement plus grand (de 10 % env.) que celui obtenu par nous, et, en conséquence, la valeur de  $k$  qu'on peut déduire de sa Fig. I est égale à env. 10; par contre, il paraîtrait que la valeur trouvée par lui pour  $\lambda_0^2$  ne s'écarte guère de la nôtre. Du reste, il ne dit rien au sujet de ses conditions expérimentales, sauf qu'il a traité la matière première à froid avec de l'alcool et de l'éther, selon la méthode de Hardy et Gardiner, et, d'autre part, qu'il a recherché le pouvoir rotatoire dans une solution dialysée, autant que possible débarrassée de sel; par conséquent, on ne saurait rien dire avec certitude sur les causes auxquelles sont imputables les différences qui existent entre ses résultats et les nôtres. En tous cas, elles ne peuvent guère, à ce qu'il paraît, provenir de ce que par suite du traitement à l'éther-alcool il y aurait eu élimination de lipoides ou de phosphatides, car il trouve le même rapport de P à N que nous. Par contre, la différence semble plutôt due en partie à une diversité de conditions expérimentales; que celles-ci soient uniquement en cause, apparaît comme quelque peu douteux.

*Mai 1927.*

COMPTES-RENDUS

DES TRAVAUX

DU

# LABORATOIRE CARLSBERG

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16<sup>ME</sup> VOLUME.

No. 11



COPENHAGUE

EN COMMISSION CHEZ H. HAGERUP

IMPRIMERIE DE THIELE

1927

*Prix: 30 Øre.*





# SUR LE POIDS SPÉCIFIQUE DES SOLUTIONS DE CHLORURE D'AMMONIUM.

PAR

HANS JESSEN-HANSEN.

Comme suite à des recherches publiées précédemment concernant le poids spécifique des solutions d'albumine renfermant du sulfate d'ammonium<sup>1)</sup>, ainsi qu'à celles également effectuées dans le Laboratoire Carlsberg, ayant trait aux solutions d'albumine contenant du chlorure d'ammonium<sup>2)</sup>, on a procédé à une série d'investigations sur le poids spécifique des solutions de chlorure d'ammonium, investigations analogues à celles antérieurement faites sur les solutions de sulfate d'ammonium<sup>3)</sup>.

Comme point de départ, nous nous sommes servi d'une solution concentrée de chlorure d'ammonium. La concentration fut déterminée en distillant et titrant à la Kjeldahl l'ammoniaque contenue dans la solution. Trois déterminations indépendantes l'une de l'autre et dont chacune était la moyenne de trois distillations, ont fait constater que 100 g de solution contenaient, respectivement:

	6g.5033 de $\text{NH}_3\text{-N}$ , écart d'avec la moyenne	+ 0.12%
	6g.4931 - — — — —	÷ 0.03%
	6g.4896 - — — — —	÷ 0.09%
Moyenne	6g.49533 de $\text{NH}_3\text{-N}$ , écart moyen	0.08%

Il en résulte que la solution contenait, par 100 g, 24g.8045 de  $\text{NH}_4\text{Cl}$  et 32g.9867 dans 100 g d'eau.

Avec cette solution on prépara par pesage et dilution convenable, des solutions correspondant aussi près que possible à 3n g de  $\text{NH}_4\text{Cl}$  dans 100 g d'eau pour toutes les valeurs en-

<sup>1)</sup> Comptes-rendus du Laboratoire Carlsberg, XVI No 10 (1927).

<sup>2)</sup> *Ibidem*, XVI No 5 (1926).

<sup>3)</sup> *Ibidem*, XII p. 153 (1917).

tières de  $n$  depuis 1 à 11, et, de plus, pour  $n = 1/3$  et  $= 2/3$ ; puis on détermina le poids spécifique que présenteraient ces solutions dans le vide à la température de 18 degrés, par rapport à l'eau de cette même température, à l'aide d'un pycnomètre de Sprengel qui pouvait contenir env. 20 g d'eau. Pour effectuer la pesée, le pycnomètre rempli de la solution fut placé dans un bain-marie à  $18^{\circ}.00 \pm 0.01$ , puis, après au moins dix minutes, le ménisque du pycnomètre fut ajusté au trait, on retira le pycnomètre de l'eau, on l'essuya et le pesa, le pesage étant terminé cinq minutes après que le pycnomètre avait été retiré du bain-marie. Le pycnomètre demeura alors pendant exactement 10 minutes sur la balance, après quoi on lut de nouveau le poids. Le résultat de cette dernière lecture était d'ordinaire un peu plus bas que celui de la première, par suite d'une évaporation émanant de la pointe du pycnomètre. La moitié de la perte observée fut ajoutée au résultat de la première pesée comme correction de l'évaporation ayant eu lieu pendant les cinq premières minutes. Lorsque cette correction s'élevait à plus de 0.02 mg, on y voyait un indice faisant soupçonner que l'essuyage n'avait pas été fait avec le soin voulu, et l'on rejeta ce résultat douteux. On observait tant la hauteur du baromètre que la température de l'armoire de la balance, et l'on ne manquait pas d'appliquer les corrections y relatives.

On répétait chaque essai et on calculait les moyennes, en faisant abstraction des valeurs où la cinquième décimale présentait un écart de plus de deux unités d'avec la moyenne; par conséquent, la différence entre la valeur la plus élevée et la plus basse ne dépasse en aucun cas le nombre de 4 de la cinquième décimale du poids spécifique. Les valeurs ainsi trouvées étaient ensuite soumises à une égalisation graphique, dont les résultats, ainsi que les moyennes des résultats expérimentaux, se trouvent inscrits au tableau I ci-contre. On apercevra que l'égalisation nécessaire ne s'élève pas, en moyenne, à plus de 1.8 unités dans la cinquième décimale, bien que dans un cas isolé elle en dépasse le double.

Nous avons regardé les valeurs égalisées comme représentant le poids spécifique réel des solutions étudiées, et c'est sur cette base que les poids spécifiques de solutions contenant  $S$  g de chlorure d'ammonium par 100 g d'eau ont été calculés par interpolation graphique pour toutes les valeurs entières de  $S$  depuis 1 à 33. Le tableau II donne ces valeurs, d  $18^{\circ}/18^{\circ}$ , en même temps que le poids spécifique des solutions à  $18^{\circ}$  par rapport à

TABLEAU I.

Grammes de chlorure d'am- monium dans 100 g d'eau	Poids spécifique		Correction $0 \div C$
	Observé	Corrigé	
32.9867 <sub>4</sub>	1.07174 <sub>5</sub>	1.07176 <sub>9</sub>	$\div 0.00002_1$
32.9547 <sub>1</sub>	1.07173 <sub>5</sub>	1.07172 <sub>11</sub>	$+$ 1 <sub>5</sub>
29.9570 <sub>1</sub>	1.06707 <sub>5</sub>	1.06705 <sub>9</sub>	$+$ 1 <sub>9</sub>
26.9721 <sub>10</sub>	1.06208 <sub>3</sub>	1.06212 <sub>2</sub>	$\div$ 3 <sub>9</sub>
23.9663 <sub>4</sub>	1.05683 <sub>1</sub>	1.05684 <sub>4</sub>	$\div$ 1 <sub>3</sub>
20.9446 <sub>4</sub>	1.05125 <sub>1</sub>	1.05121 <sub>9</sub>	$+$ 3 <sub>2</sub>
17.9716 <sub>8</sub>	1.04532 <sub>5</sub>	1.04535 <sub>9</sub>	$+$ 2 <sub>4</sub>
14.9766 <sub>6</sub>	1.03912 <sub>2</sub>	1.03909 <sub>9</sub>	$+$ 2 <sub>3</sub>
11.9861 <sub>1</sub>	1.03244 <sub>2</sub>	1.03244 <sub>3</sub>	$\div$ 0 <sub>1</sub>
8.9893 <sub>4</sub>	1.02529 <sub>1</sub>	1.02529 <sub>5</sub>	$\div$ 0 <sub>7</sub>
5.9888 <sub>3</sub>	1.01757 <sub>7</sub>	1.01759 <sub>5</sub>	$\div$ 1 <sub>8</sub>
2.9960 <sub>7</sub>	1.00928 <sub>2</sub>	1.00927 <sub>9</sub>	$+$ 0 <sub>3</sub>
1.9963 <sub>9</sub>	1.00628 <sub>3</sub>	1.00628 <sub>5</sub>	$\div$ 0 <sub>5</sub>
1.0002 <sub>5</sub>	1.00317 <sub>3</sub>	1.00320 <sub>5</sub>	$\div$ 3 <sub>2</sub>
0.9992 <sub>5</sub>	1.00322 <sub>4</sub>	1.00320 <sub>2</sub>	$+$ 2 <sub>2</sub>

l'eau à 4°, d 18°/4°, ainsi que le volume à 18°,  $V_s$ , de 100 g d'eau + S g de sel, et enfin la concentration  $c = \frac{S \cdot 1000}{V_s \cdot 53.512}$ .

Il sera peut-être d'un certain intérêt de comparer ces valeurs avec celles auxquelles d'autres chercheurs sont arrivés. Parmi les recherches qui me sont connues au sujet du poids spécifique de solutions de chlorure d'ammonium, celles faites par D. Dijken<sup>1)</sup> paraissent exécutées avec une précision toute particulière. Elles ne se rapportent toutefois qu'à des solutions très diluées, et de ce fait, il n'y a, à proprement parler, que trois des valeurs de Dijken qui puissent être comparées avec les miennes. Dijken détermine le poids spécifique à 16°, et la concentration, en grammes de sel par 1000 g de solution. Le tableau III ci-dessous montre les données originales de Dijken, ainsi que la concentration convertie en grammes de sel par 100 g d'eau et le poids spécifique converti en 18°/18°; l'augmentation de volume de la solution de chlorure d'ammonium étant calculée d'après la formule

$$V_{18} = V_{16} (1 + 2 \times 0.00022)^2,$$

<sup>1)</sup> Zeitschrift für physik. Chemie, **24** 107 (1897).

<sup>2)</sup> C. Bender: Wiedem. Ann., **31**, 872 (1887). Citées d'après Landolt et Börnstein Tabellen. 4 Aufl. 1912, p. 339.

TABLEAU II.

Poids spécifique de solutions de chlorure d'ammonium.

$S = \text{g NH}_4\text{Cl dans } 100 \text{ g d'eau}$   $V_s = \text{volume à } 18^0 \text{ de la quantité,}$   
 en poids, de solution contenant 100 g d'eau.  $c = \text{concentration}$

$$\text{équivalente à } 18^0 = \frac{1000 S}{V_s \cdot 53.502}.$$

S	Poids spécifique		$V_s$	c
	$18^0/18^0$	$18^0/4^0$		
33.0000	1.07178 <sub>9</sub>	1.07031 <sub>2</sub>	124.2628	4.964
32	1.07026 <sub>9</sub>	1.06879 <sub>4</sub>	123.5037	4.843
31	1.06871 <sub>6</sub>	1.06724 <sub>3</sub>	122.7462	4.720
30	1.06712 <sub>9</sub>	1.06565 <sub>8</sub>	121.9904	4.596
29	1.06550 <sub>9</sub>	1.06404 <sub>1</sub>	121.2359	4.471
28	1.06385 <sub>6</sub>	1.06239 <sub>0</sub>	120.4831	4.343
27	1.06217 <sub>0</sub>	1.06070 <sub>6</sub>	119.7316	4.215
26	1.06044 <sub>8</sub>	1.05898 <sub>7</sub>	118.9816	4.084
25	1.05869 <sub>3</sub>	1.05723 <sub>4</sub>	118.2330	3.952
24	1.05690 <sub>8</sub>	1.05544 <sub>9</sub>	117.4855	3.818
23	1.05508 <sub>0</sub>	1.05362 <sub>6</sub>	116.7397	3.682
22	1.05322 <sub>0</sub>	1.05176 <sub>9</sub>	115.9951	3.545
21	1.05132 <sub>5</sub>	1.04987 <sub>6</sub>	115.2517	3.406
20	1.04939 <sub>4</sub>	1.04794 <sub>8</sub>	114.5095	3.264
19	1.04742 <sub>5</sub>	1.04598 <sub>2</sub>	113.7687	3.121
18	1.04541 <sub>7</sub>	1.04397 <sub>6</sub>	113.0294	2.977
17	1.04336 <sub>8</sub>	1.04193 <sub>0</sub>	112.2906	2.830
16	1.04127 <sub>9</sub>	1.03984 <sub>4</sub>	111.5552	2.681
15	1.03915 <sub>0</sub>	1.03771 <sub>8</sub>	110.8201	2.530
14	1.03697 <sub>0</sub>	1.03554 <sub>1</sub>	110.0874	2.377
13	1.03474 <sub>5</sub>	1.03331 <sub>9</sub>	109.3564	2.222
12	1.03247 <sub>5</sub>	1.03105 <sub>2</sub>	108.6269	2.065
11	1.03014 <sub>4</sub>	1.02872 <sub>4</sub>	107.9007	1.905
10	1.02776 <sub>1</sub>	1.02634 <sub>5</sub>	107.1764	1.744
9	1.02532 <sub>5</sub>	1.02391 <sub>2</sub>	106.4545	1.580
8	1.02282 <sub>0</sub>	1.02141 <sub>1</sub>	105.7361	1.414
7	1.02025 <sub>3</sub>	1.01884 <sub>7</sub>	105.0207	1.246
6	1.01762 <sub>5</sub>	1.01622 <sub>3</sub>	104.3078	1.075
5	1.01493 <sub>4</sub>	1.01353 <sub>5</sub>	103.5978	0.902
4	1.01215 <sub>5</sub>	1.01076 <sub>0</sub>	102.8929	0.727
3	1.00928 <sub>8</sub>	1.00789 <sub>7</sub>	102.1930	0.549
2	1.00629 <sub>9</sub>	1.00491 <sub>2</sub>	101.5014	0.368
1	1.00320 <sub>4</sub>	1.00182 <sub>1</sub>	100.8164	0.185
0	1.00000 <sub>0</sub>	0.99862 <sub>2</sub>	100.1380	

TABLEAU III.

Concentration		Poids spécifique			Différence a ÷ b 0,0000
Dijken g dans 1000 g de solution	Converti en g de chlorure d'ammonium dans 100 g d'eau	Dijken trouvé 16° : 16°	Converti en 18° : 18° a	Interpolé d'après tableau II b	
0.4431	0.0443	1.000150	1.00006 <sub>1</sub>	1.00014 <sub>1</sub>	÷ 80
0.9061	0.0907	1.000340	1.00021 <sub>3</sub>	1.00029 <sub>0</sub>	÷ 77
1.8085	0.1812	1.000606	1.00051 <sub>5</sub>	1.00058 <sub>0</sub>	÷ 65
3.5947	0.3608	1.001196	1.00110 <sub>5</sub>	1.00115 <sub>4</sub>	÷ 49
7.7845	0.7845	1.002562	1.00247 <sub>1</sub>	1.00251 <sub>0</sub>	÷ 39
15.3425	1.5582	1.004994	1.00492 <sub>6</sub>	1.00492 <sub>5</sub>	+ 01
31.2364	3.2244	1.010018	1.00992 <sub>6</sub>	1.00992 <sub>9</sub>	÷ 03

et l'augmentation de volume de l'eau étant évaluée à  $\frac{998970}{998622} = 1.000349^1$ ); donc, le poids spécif.  $18^\circ/18^\circ = P_s \cdot 16^\circ/16^\circ$  divisé par  $\frac{1.00044}{1.000349}$

$$= \frac{P_s \cdot 16^\circ/16^\circ}{1.000091}$$

On voit qu'il y a une excellente concordance entre les valeurs dérivées des résultats de Dijken et les miennes pour ce qui concerne les deux solutions les plus concentrées de Dijken. Pour les solutions plus diluées, l'accord est, à ce qu'il paraît, sensiblement moins bon, ce qui probablement s'explique par ce fait, que la valeur admise pour le coefficient de dilatation des solutions de chlorure d'ammonium, soit 0.00022, est celle qui s'applique à une solution à 5.3%, tandis que pour les solutions plus concentrées elle est plus élevée (par exemple, pour 24.9% = 0.00036) et pour les moins concentrées indubitablement plus basse<sup>2)</sup>.

<sup>1)</sup> Börnstein et Landolt Tabellen, 4 Aufl., p. 43.

<sup>2)</sup> Il va de soi que le coefficient de dilatation des solutions très diluées se rapproche de celui de l'eau, de sorte que  $P_s \cdot 16^\circ/16^\circ$  se rapproche de  $P_s \cdot 18^\circ/18^\circ$ , et conformément à cela, on remarque que, pour les trois solutions les moins concentrées, les valeurs trouvées par moi pour  $P_s \cdot 18^\circ/18^\circ$  sont beaucoup plus proches des valeurs de Dijken pour  $P_s \cdot 16^\circ/16^\circ$  qu'elles ne le sont de celles réduites par le calcul indiqué plus haut.

La concordance ainsi constatée entre les résultats de Dijken et les miens, me semble confirmer encore l'exactitude des uns et des autres, du moins avec le degré de précision que j'ai tâché d'atteindre, c. à d. que l'erreur éventuelle serait inférieure à 2 unités de la 5<sup>ème</sup> décimale.

*Mars 1927.*

COMPTES-RENDUS

DES TRAVAUX

DU

# LABORATOIRE CARLSBERG

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16<sup>ME</sup> VOLUME.

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# ÉTUDES SUR LES PROTÉINES

PAR

S. P. L. SØRENSEN.

## XI. SUR LA TENEUR EN PHOSPHORE ET LA SOLUBILITÉ DE L'OVALBUMINE

PAR

M. MÂCHEBŒUF, MARGRETHE SØRENSEN ET S. P. L. SØRENSEN.

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## 1<sup>o</sup>. Aperçu de la teneur en phosphore et de la solubilité des albumines et des globulines.

Pour la préparation de l'albumine d'œuf ou de sérum cristallisée, on emploie en général le procédé indiqué par F. G. Hopkins et S. N. Pinkus<sup>1)</sup>, d'après lequel le blanc d'œuf ou le sérum bruts sont mélangés avec un volume égal d'une dissolution saturée de sulfate d'ammonium, la globuline étant ainsi précipitée, tandis que pour l'albumine on peut la faire précipiter de la liqueur filtrée ou bien l'en faire cristalliser au moyen d'une addition supplémentaire de solution d'ammonium et d'une acidulation convenable par de l'acide sulfurique dilué. Les globulines et albumines ainsi obtenues peuvent être purifiées par lavage avec des solutions de sulfate d'ammonium de concentration convenable, puis dissolution dans l'eau et précipitation ou cristallisation renouvelées.

Les substances purifiées par des précipitations ou des cristallisations répétées, renferment toutes de petites quantités de phosphore; cependant, il existe une différence caractéristique entre les produits fournis par le blanc d'œuf et ceux que donne le sérum de cheval, car la globuline d'œuf contient, par gramme d'azote, moins de phosphore que l'ovalbumine<sup>2)</sup>, alors que pour la globuline et l'albumine provenant du sérum c'est l'inverse<sup>3)</sup>.

Il est naturel de penser qu'il existe une relation entre la différence de solubilité de ces substances, d'un côté, et, de l'autre, leur teneur différente en phosphore, et en vue d'éclaircir cette relation nous avons, en ce qui concerne les albumine et globuline de sérum, rassemblé de copieux matériaux pendant le courant de nombreuses années. Ces investigations, dont les résultats n'ont été publiés qu'en partie jusqu'ici<sup>4)</sup>, ont montré que l'albumine de sérum se laisse séparer, par cristallisation fractionnée, en fractions possédant des teneurs différentes en phosphore et des solubilités différentes dans les solutions de sulfate d'ammonium, et nous avons

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<sup>1)</sup> Journ. of Physiol., **23**, 130 (1898).

<sup>2)</sup> Thomas B. Osborne and George F. Campbell: Journ. Americ. Chem. Soc., **22**, 428 (1900).

<sup>3)</sup> S. P. L. Sørensen: Compt. rend. Laboratoire Carlsberg, **16**, No 8, page 16 (1926); Journ. Chem. Soc. 1926, page 3006.

<sup>4)</sup> Compt. rend. Laboratoire Carlsberg, **15**, No 11 (1925), **16**, No 8 (1926); Journ. Americ. Chem. Soc. **47**, 457 (1925); S. P. L. Sørensen, Proteins, Lectures given in the United States of America in 1924 (The Fleischmann Laboratories), p. 1 and 40.

pu constater que plus la teneur en phosphore est petite, plus la solubilité est faible. Les globulines de sérum, elles aussi, ont pu être fractionnées, soit par dialyse — procédé dont nous ferons abstraction ici — soit par précipitation fractionnée au moyen du sulfate d'ammonium. Les fractions de globuline obtenues par ce dernier procédé accusaient également des teneurs variables en phosphore et des solubilités variables dans les solutions de sulfate d'ammonium, — en ce sens cependant que la solubilité se trouvait d'autant moindre, que la teneur en phosphore était plus forte. Ainsi donc, il ne semble pas exister de corrélation générale entre le taux de phosphore et la solubilité: les résultats expérimentaux rapportés ici donnent plutôt, paraît-il, à penser que nous avons affaire à de faibles doses d'impuretés phosphorées, manière de voir qui, en tout cas pour ce qui concerne l'euglobuline, est appuyée par les recherches de Harriette Chick<sup>1)</sup>.

Avec le projet de nous renseigner, si possible, sur la question de savoir quel est le genre de liaison observée entre les substances protéiques du sérum et les éléments renfermant du phosphore, nous avons cru devoir, à côté de la détermination ordinaire du «phosphore coagulable»<sup>2)</sup>, introduire une évaluation du «phosphore précipitable par l'alcool», évaluation en vue de laquelle la matière protéique est coagulée au moyen de l'alcool et d'un chauffage subséquent (pour les détails du mode opératoire, nous renvoyons à la page 17). Pour autant que les constituants phosphorés seraient bien réellement fortement liés à la substance protéique, il faudrait s'attendre, aussi bien pour la coagulation par l'alcool que lors de la coagulation usuelle par la chaleur, à les voir suivre la protéine. Dans le cas, au contraire, où il ne s'agirait que de lécithine adsorbée ou d'autres corps phosphorés analogues faiblement fixés, il y a lieu d'admettre qu'en tous cas une grande partie de ces corps demeureront dans l'eau-mère alcoolique.

Or, pour ce qui regarde les protéines du sérum, nous avons pu établir qu'une bien faible partie seulement, soit deux ou trois pour cent, du phosphore coagulable par la chaleur est précipitable par l'alcool, la plus grande partie de ce phosphore restant

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<sup>1)</sup> Biochem. Journ., **8**, 404 (1914).

<sup>2)</sup> Margrethe Sørensen: Compt. rend. Laboratoire Carlsberg, **15**, N° 10 (1925).

dans l'eau-mère alcoolique. Cette constatation semble justifier la conclusion que la partie de beaucoup la plus importante des constituants phosphorés du sérum ne sont probablement liés aux protéines du sérum que d'une façon assez faible. Si nous ne tenons pas cette conclusion pour absolument sûre, c'est parce que dans la détermination du phosphore précipitable par l'alcool il se produit une dénaturation des protéines, et il est toujours possible que cette dénaturation soit justement accompagnée, peut-être même conditionnée, par une séparation d'éléments phosphorés. La preuve serait bien plus certaine, si l'on pouvait parvenir à extraire du sérum, ou à en éliminer d'une autre manière quelconque, les constituants phosphorés, sans que les principes protéiques en subissent une dénaturation ni aucune autre modification de leurs propriétés caractéristiques.

Le traitement du sérum avec l'éther à la température de la glacière, ne conduira pas, ou en tout cas ne conduira qu'en partie, au but visé, car s'il est vrai que — comme il sera mentionné dans un mémoire ultérieur — cette manière de faire n'a pas pour conséquence de dénaturer les protéines ni de les altérer d'aucune autre façon, toujours est-il qu'elle n'aboutit qu'à éliminer une partie du phosphore coagulable.

Nous avons été ainsi conduits à utiliser un autre procédé, imaginé par W. B. Hardy et M<sup>me</sup> S. Gardiner<sup>1)</sup> et employé plus tard par E. G. Young<sup>2)</sup>, procédé qui s'est montré beaucoup plus convenable au but: On précipite le sérum à  $\div 4^0$  à l'aide de l'alcool, puis, après avoir séparé le précipité par filtration, on le lave soigneusement à la même température avec de l'alcool et de l'éther, après quoi on le débarrasse de la dernière trace d'alcool par extraction au moyen d'éther dans des appareils de Soxhlet, pour lui enlever finalement l'éther par abandon dans le vide sur l'acide sulfurique. De cette façon on arrive à éliminer la presque-totalité du phosphore coagulable, sans faire perdre aux protéines leurs propriétés caractéristiques. Le précipité débarrassé de l'éther se montre complètement soluble dans l'eau, et de la dissolution aqueuse les globulines peuvent être précipitées, comme d'habitude, par demi-saturation avec le sulfate d'ammonium; de même que de la liqueur filtrée provenant du précipité de globuline

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<sup>1)</sup> Journ. Physiol., **40**, 68 (1910).

<sup>2)</sup> Proc. Roy. Soc. London B., **93**, 15 (1922).

on peut précipiter l'albumine à l'état cristallin par une acidulation convenable.

Il se pose maintenant la question de savoir si les globulines et albumines ainsi purifiées, dénuées de phosphore ou en tout cas très pauvres en cet élément, offrent des conditions de fractionnement et des solubilités identiques, ou du moins sensiblement pareilles, à celles des substances analogues tirées directement du sérum. Jusqu'à ce jour, nous n'avons réalisé que quelques essais préliminaires sur l'albumine et l'euglobuline, essais dans lesquels nous avons rencontré des solubilités qui, tout au moins qualitativement, correspondent à celles des substances phosphorées analogues. Ainsi, il paraîtrait que les constituants phosphorés des protéines sériques obtenues de la façon habituelle doivent être envisagés comme des impuretés, qui n'ont aucun rapport aux propriétés caractéristiques des protéines et qui sans doute n'influencent pas d'une façon essentielle leur solubilité ni leur précipitation. Pour pouvoir donner une réponse définitive à cette dernière question, il faut cependant des données expérimentales plus étendues que celles dont nous disposons actuellement; cependant, des essais dans ce sens sont commencés et, aussitôt qu'ils seront achevés, l'ensemble de nos matériaux relatifs aux solubilités et aux conditions de précipitation des albumines et globulines de sérum sera publié.

Les divers faits brièvement résumés ci-dessus concernant les constituants phosphorés des protéines sériques, soulèvent tout naturellement la question de savoir, quel est le rôle des substances phosphorées de l'albumine d'œuf. Est-ce qu'ici encore nous sommes simplement en présence d'impuretés, ou bien le phosphore entre-t-il comme partie intégrante dans la molécule ou le complexe moléculaire de l'ovalbumine? Les recherches relatées dans le présent mémoire ont précisément pour but de jeter de la lumière sur cette question.

## 2. Teneur en phosphore de l'ovalbumine.

Il est connu depuis longtemps — surtout par les investigations très approfondies de Thomas B. Osborne et George F. Campbell<sup>1)</sup> — que l'albumine d'œuf renferme des quantités faibles mais cependant facilement dosables de phosphore (soit

<sup>1)</sup> Journ. Americ. Chem. Soc., **22**, 422 (1900).

0.112 à 0.131 %, calculé sur la matière sèche dénuée de cendres). Pour ce qui regarde la manière dont ce phosphore est probablement liée à la substance protéique, les auteurs estiment<sup>1)</sup> que "it seems highly probable that this phosphorus belongs to an acid united with the crystallized albumin in the same manner as the writer has shown that hydrochloric acid unites with edestin to form crystalline compounds. As to the nature of this phosphorized acid we have learned nothing, owing to the small proportion in which it is present".

Karl Kaas<sup>2)</sup>, sans connaître les travaux d'Osborne et Campbell, a exécuté quelques années plus tard, dans le laboratoire de Skraup à Graz, une série de dosages de phosphore de l'albumine d'œuf, et il est arrivé à des résultats bien singuliers.

Dans un échantillon d'ovalbumine, Kaas a constaté la présence de 0.919 % de phosphore, alors que dans un autre il en a trouvé 3.06 %, chiffre qui a toutefois diminué à la suite d'une reprécipitation répétée, à tel point que la septième fraction s'est trouvée ne renfermer que 1.73 % de phosphore. Un échantillon d'albumine brute provenant d'un œuf frais pondu ne présentait que 0.155 % de cet élément, tandis qu'un échantillon analogue prélevé sur un œuf âgé d'un mois en accusait 0.228 %. Enfin, l'auteur examina deux échantillons d'ovalbumine que lui avait cédés M. Ludwig de Vienne; dans l'un d'eux, il ne put déceler le phosphore, dans l'autre il en trouva 0.352 %.

À propos des résultats analytiques que nous venons de citer, Skraup fait remarquer qu'ils paraissent prouver péremptoirement que l'ovalbumine cristallisée renferme très souvent du phosphore, en quantité variable suivant la race de poule et son régime alimentaire. Il se peut sans doute que Skraup ait raison; pourtant, à notre connaissance, aucun autre chercheur n'a constaté, dans la teneur en phosphore de l'albumine d'œuf, des variations aussi considérables que celles dont il est question ici, de même que dans la littérature qui nous est accessible il ne se trouve pas d'indication d'une proportion de phosphore si extrêmement élevée qu'elle atteindrait ou même dépasserait 1 %.

Quoiqu'il en soit, il faut dire que cette question n'a été l'objet jusqu'ici que de rares recherches. En dehors de celles

<sup>1)</sup> *loc. cit.*, page 443.

<sup>2)</sup> Sitzungsberichte der Akademie der Wissenschaften (Wien), **115**, Abt. II b, 231 (1906).

mentionnées ci-dessus, nous n'avons en effet pu trouver qu'une courte notice de M<sup>lle</sup> E. G. Willcock et W. B. Hardy<sup>1)</sup> traitant la question de la teneur en phosphore de l'albumine d'œuf cristallisée. Dans sept différents échantillons d'ovalbumine, préparés et recristallisés de manières variées, ces auteurs signalent la présence de 0.126 à 0.140 % de phosphore, chiffres très voisins de ceux indiqués par Osborne et Campbell. Willcock et Hardy sont d'avis que le phosphore fait partie intégrante de la molécule d'ovalbumine, car ils ont vérifié que, dénaturée par la cuisson ou par un traitement à l'alcool, l'albumine contenait toujours du phosphore, que l'on ne put éliminer par un traitement à l'alcool ou à l'éther, ni même par un traitement subséquent à l'acide acétique à 50 % ou encore au sel marin en solution légèrement acide. Cette opinion n'a cependant jamais été généralement approuvée<sup>2)</sup>; dans bon nombre de manuels et de traités de chimie, il n'est même pas mentionné que l'ovalbumine cristallisée et purifiée renferme du phosphore, sans doute parce que les auteurs supposent implicitement qu'une teneur éventuelle provient d'impuretés.

Dans les expériences effectuées pendant ces dernières années dans notre Laboratoire Carlsberg, touchant la question qui nous occupe ici, nous avons, dans tous les échantillons d'ovalbumine cristallisée, fraîchement préparés et bien purifiés, trouvé une proportion sensiblement constante de phosphore, comprise entre 0.111 et 0.123 % (moyenne 0.117 %), donc très légèrement inférieure à celle indiquée par Osborne et Campbell. Nous indiquons habituellement cette proportion d'une façon un peu différente, qui sera employée constamment dans la suite, savoir en milligrammes de phosphore par gramme d'azote d'albumine. Si on pose égal à 6.4 le facteur<sup>3)</sup> par lequel il faut multiplier le poids d'azote d'ovalbumine pour obtenir le poids d'ovalbumine sèche, la proportion d'azote sus-indiquée correspondra à 7<sup>mg</sup>.1 à 7<sup>mg</sup>.9 de phosphore par gramme d'azote d'albumine (moyenne 7<sup>mg</sup>.5).

Comme, d'autre part, nous n'avons pu remarquer de différence entre la quantité de «phosphore total» et celle de «phos-

<sup>1)</sup> Proc. Cambridge Philos. Soc., **14**, 119 (1907).

<sup>2)</sup> Voir toutefois S. P. L. Sørensen et Margrethe Høyrup: Compt. rend. Laboratoire Carlsberg, **12**, 18 note 2 (1916).

<sup>3)</sup> S. P. L. Sørensen et Margrethe Høyrup: Compt. rend. Laboratoire Carlsberg, **12**, 181 (1917).

phore coagulable» ou de «phosphore précipitable par l'alcool» (v. p. 16), il nous semble raisonnable d'admettre, à l'instar de Willcock et Hardy, que l'albumine d'œuf, contrairement à celle de sérum, contient comme élément intégrant une substance phosphorée. On se demandera alors si cette proportion très infime de phosphore peut cadrer avec nos vues actuelles sur la grandeur de la molécule d'ovalbumine.

Dans quelques travaux publiés il y a une dizaine d'années touchant l'albumine d'œuf, nous avons pensé, en nous fondant sur des mesures faites de la pression osmotique de solutions de cette albumine, pouvoir estimer à environ 34000 le poids de la molécule d'ovalbumine ou du complexe de molécules à l'état anhydre, d'où la proportion d'atomes d'azote s'évalue à 380 env.<sup>1)</sup>. Cette grandeur de molécule s'accorde parfaitement avec le poids moléculaire admis par Edwin J. Cohn (33200), qui supposait l'existence de deux groupes de tryptophane dans la molécule<sup>2)</sup>, — et aussi avec le poids moléculaire trouvé par I. B. Nichols (34500) pour l'ovalbumine bien purifiée, au moyen de la méthode de centrifugation de Svedberg<sup>3)</sup>.

Si donc nous évaluons à 34000 le poids moléculaire de l'ovalbumine et à 380 le nombre des atomes d'azote, un simple calcul donnera ce résultat, que 1 atome de phosphore dans cette molécule demande une teneur de 5<sup>mg</sup>.8 en phosphore par g d'azote, puisque

$$\frac{31 \cdot 1000}{380 \cdot 14} = 5.8$$

L'ordre de grandeur de la proportion de phosphore trouvée va donc bien avec la supposition d'un atome de phosphore dans la molécule d'ovalbumine. Cependant, d'un autre côté, 7.5 est trop supérieur à 5.8 pour que cette différence puisse s'expliquer par des erreurs analytiques. Comme il a été brièvement mentionné à la troisième réunion des chimistes scandinaves à Helsingfors, en juillet 1926<sup>4)</sup>, nous avons essayé de diverses manières s'il ne serait pas possible de réduire la teneur en phos-

<sup>1)</sup> Compt. rend. Laboratoire Carlsberg, **12**, 356 (1917).

<sup>2)</sup> Physiol. Reviews V, N° 3, 359 (1925).

<sup>3)</sup> Zeitschr. physik. Chem., **121**, 76 (1926), et Journ. Americ. Chem. Soc., **48**, 3081 (1926).

<sup>4)</sup> Compt. rend. Laboratoire Carlsberg, **16** N° 8 (1926).



phore de l'ovalbumine, sans faire perdre à la substance sa faculté caractéristique de cristallisation bien marquée.

Les essais dont les détails se trouvent rapportés dans la partie expérimentale (Section B) du présent mémoire, feront voir:

1°. Que par une cristallisation fractionnée à des concentrations variées en ions hydrogène, nous ne sommes pas parvenus à préparer des fractions à teneurs différentes en phosphore, ces teneurs ne variant dans les diverses fractions que de 7<sup>mg</sup>.52 à 7<sup>mg</sup>.78 par g d'azote d'albumine (Section B. a.).

2°. Qu'une diminution de la quantité de phosphore n'a pas pu être obtenue non plus par un traitement à l'ammoniaque pendant vingt-quatre heures à la température ordinaire ( $p_{\text{NH}}$  étant égal à 9.7 environ), traitement qui était suivi d'une neutralisation de l'ammoniaque et de la séparation de l'albumine par cristallisation à la manière habituelle (Section B. b.)

3°. Que par la précipitation à basse température, au moyen de l'alcool, d'une solution d'ovalbumine, puis lavage avec l'alcool et l'éther, on n'a pas réussi à extraire de l'albumine des matières contenant du phosphore; ce traitement a eu pour effet une dénaturation de l'albumine qui, après comme avant, s'est montrée renfermer, par g d'azote, environ 7<sup>mg</sup>.5 de phosphore (Section B. c.).

4°. Qu'à la suite d'une électrodialyse opérée dans l'appareil de Pauli tel qu'il est ordinairement employé<sup>1)</sup>, et dans lequel la solution d'ovalbumine était placée entre deux membranes de collodion, l'on a observé que, la liqueur cathodique demeurant exempte d'albumine durant plusieurs jours, une partie de l'albumine passait dans la liqueur anodique, et cette albumine offrait une teneur en phosphore légèrement supérieure à celle de la matière dont on était parti, tandis que pour l'albumine qui était restée dans la liqueur intérieure c'était l'inverse (Section B. d.).

5°. Que des solutions d'ovalbumine dialysées et conséquemment pauvres en électrolyte, qui avaient été abandonnées en glacière depuis plusieurs années, avaient déposé à l'état dénaturé une partie de l'albumine, et que le reste, qui cristallisait facilement et très nettement, a accusé une teneur en phosphore inférieure au taux normal, soit 4<sup>mg</sup>.7 à 6<sup>mg</sup>.7 par g. d'azote d'albumine (Section B. e.).

De ces données expérimentales il ressort que le complexe

<sup>1)</sup> Biochem. Zeitschr., **152**, 357 (1924).

phosphoré faisant partie de la molécule d'ovalbumine ne saurait en être séparé qu'avec difficulté. En outre, il convient de faire remarquer que — bien qu'il ait été possible de préparer des échantillons nettement cristallisés ayant une teneur en phosphore inférieure à la normale ou même inférieure à celle correspondant à 1 atome de phosphore dans la molécule —, nous n'avons jamais eu entre les mains des échantillons d'ovalbumine cristallisée contenant des doses aussi minimes que celles rencontrées dans des échantillons d'albumine sérique purifiée. Pour tous nos échantillons d'ovalbumine, la quantité de phosphore a toujours été sensiblement voisine de celle correspondant à 1 atome de phosphore pour 380 atomes d'azote d'albumine.

Nous pensons donc pouvoir formuler comme suit la conclusion principale de cette partie de nos recherches:

Il doit être considéré comme très vraisemblable que, parmi les complexes dont est composée la grande molécule d'ovalbumine, il s'en trouve un contenant du phosphore et qui est si fermement lié aux autres groupes composant la molécule qu'il faut le regarder, comme une partie intégrante de celle-ci.

### 3. Solubilité de l'ovalbumine.

La deuxième question principale dont s'occupe ce travail, se rapporte à l'état d'équilibre existant entre l'albumine d'œuf cristallisée et l'eau-mère qui entoure le précipité; en d'autres termes, il sera question ici de la solubilité de l'ovalbumine cristallisée dans des dissolutions concentrées de sulfate d'ammonium. Tout d'abord, il s'agit de résoudre la question de savoir si la solubilité de l'ovalbumine dans des conditions données peut être regardée comme constante et, plus spécialement, si la solubilité est indépendante de la teneur en phosphore de l'échantillon d'ovalbumine examiné.

Déjà dans les «Études sur les Protéines» IV<sup>1)</sup> les solubilités de l'ovalbumine dans les dissolutions concentrées de sulfate d'ammonium ont été étudiées d'une façon approfondie, et il y est montré comment la vitesse de cristallisation aussi bien que l'état d'équilibre dépendent des circonstances (température, concentration en sulfate d'ammonium, en ions hydrogène et en matières

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<sup>1)</sup> Compt. rend Laboratoire Carlsberg, **12**, 213 (1917).

protéiques). Les résultats des expériences que nous avons exécutées depuis, sont venus confirmer entièrement ceux obtenus alors. C'est ainsi que, par exemple, nous retrouvons dans les expériences nouvelles la grande influence qu'exerce sur la solubilité une modification de la concentration en ions hydrogène de l'eau-mère. A ce sujet, celles relatées dans la suite (page 21) feront voir que la solubilité se trouvera plus que doublée lorsque la concentration en ions hydrogène de l'eau-mère sera modifiée de 0.2 dans la valeur de  $p_{aH}$ ; en tout cas, ceci est vrai dans les limites de la concentration ionique adoptée dans ces expériences. De cette constatation il résulte immédiatement qu'une comparaison entre les solubilités de différents échantillons d'ovalbumine ne sera possible qu'à la condition que l'activité des ions hydrogène de l'eau-mère provenant des divers échantillons soit mesurée bien exactement et que, pour des différences possibles constatées dans ladite activité, on appliquera aux valeurs trouvées de la solubilité des corrections correspondantes. La meilleure marche à suivre se trouve exposée à la p. 21.

C'est ici le lieu de mentionner brièvement l'influence exercée sur la solubilité par la concentration protéique. Nos anciennes recherches, publiées en 1917, paraissaient justifier cette conclusion, que la concentration initiale en protéines n'a guère d'influence sur l'état d'équilibre du système, puisque les écarts observés d'avec cette règle ne sont que d'une importance secondaire et s'expliquent facilement et d'une manière très naturelle, de telle sorte que le système examiné paraît, au fond, se comporter comme un système obéissant à la règle des phases formulée par Gibbs et ne contenant qu'une seule phase solide, et formé des quatre composants: eau, hydrate d'œuf, ammoniacque et acide sulfurique. Les expériences effectuées depuis cette époque dans notre laboratoire, ont pleinement confirmé les résultats obtenus alors, attendu qu'il a été vérifié que la quantité d'hydrate d'œuf contenue dans l'eau-mère, n'est pas complètement indépendante de la concentration initiale des protéines, étant plus grande si la concentration protéique initiale est plus forte. Pour expliquer ce fait expérimental, nous avons précédemment<sup>1)</sup> insisté sur la tendance manifestée par l'ovalbumine cristallisable à se convertir, par le repos, en albumine non cristallisable mais

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<sup>1)</sup> *loc. cit.*, p. 255.

totalelement coagulable. Néanmoins, vu les expériences acquises dans nos investigations plus récentes et qui ont fait constater que, grâce à une précipitation ou une cristallisation fractionnée convenable, il est bien possible de séparer les protéines en fractions ayant des solubilités différentes, nous sommes maintenant portés à interpréter le fait en question comme un fractionnement de l'albumine. Cette manière de voir est appuyée par les expériences rapportées dans la section B a, lesquelles ont permis de constater une séparation de l'albumine en fractions accusant nettement une différence — pas très grande, il est vrai — de solubilité.

Le fait qu'un tel fractionnement peut s'opérer, tient selon nous à ce que la molécule d'ovalbumine est formée par un certain nombre de complexes réunis d'une façon relativement faible et qui, tout en n'étant point tout à fait identiques les uns aux autres, se ressemblent au point d'être partiellement remplaçables les uns par les autres dans la molécule protéique, sans que la faculté de cristallisation de l'albumine en soit annihilée. Dans une cristallisation fractionnée, la combinaison de complexes donnant le produit le plus difficilement soluble, sera alors celle qu'on verra cristalliser la première.

Lorsqu'il s'agit de la question qui nous occupe ici, l'estimation de la solubilité d'un échantillon d'ovalbumine donné, le fait expérimental que nous venons de signaler est d'une certaine importance, en ce sens que pour une détermination bien précise de la solubilité il faut absolument tenir compte de la concentration initiale en matières protéiques, et en établissant le procédé à employer pour les analyses nous avons donc été obligés de prendre en considération ce fait aussi. La Section A (p. 17) donne un exposé très détaillé du mode opératoire choisi pour la détermination de la solubilité.

Nous n'entrerons pas ici dans des considérations théoriques complémentaires sur le caractère de l'état d'équilibre dont il s'agit. La question de la précipitation de substances protéiques par des sels a été traitée à fond, il y a deux ans, par Edwin J. Cohn<sup>1)</sup>, et il a aussi étudié la précipitation et la cristallisation de l'albumine d'œuf à l'aide du sulfate d'ammonium. S'appuyant sur des expériences exécutées en partie à l'Institut Lister à Londres et en partie au Laboratoire Carlsberg, il estime que la relation

<sup>1)</sup> Physiological Reviews V, 410 (1925).

existant entre la solubilité de l'ovalbumine et la concentration du sulfate d'ammonium peut s'exprimer, dans des conditions égales d'ailleurs, par une simple fonction linéaire :

$$\log s = a \cdot m + \beta$$

où  $s$  représente la solubilité,  $m$  la concentration molaire en sulfate d'ammonium,  $a$  et  $\beta$  étant des constantes. Des modifications subies par la concentration en ions hydrogène n'ont pas pour effet de faire varier  $a$ , mais se font sentir par des modifications appréciables de la valeur de  $\beta$ . D'après Cohn, une manière de voir semblable peut s'appliquer à la précipitation d'autres protéines par des sels, et à l'appui de son opinion il cite une série d'expériences effectuée au Laboratoire Carlsberg sur la pseudoglobuline tirée du sérum de cheval. Les nombreuses déterminations de solubilité de l'albumine et de la globuline de sérum faites dans ces dernières années dans notre laboratoire peuvent également, en grande partie, être comprises dans l'idée émise par Cohn; pourtant, c'est seulement dans le cas de l'albumine d'œuf que nous avons trouvé la fonction linéaire valable pour l'ensemble des concentrations de sels examinées.

En conformité des désignations de concentrations habituellement employées par nous, nous ferons usage de l'équation de Cohn sous la forme suivante :

$$\log s = a \cdot S + \beta \quad (1)$$

où  $s$  et  $S$  signifient respectivement la quantité d'hydrate d'œuf et de sulfate d'ammonium en grammes par 100 g d'eau de l'eau-mère.

Afin de déterminer les valeurs de  $a$  et de  $\beta$  sous les conditions normales indiquées dans la partie expérimentale de ce mémoire (p. 21), on a effectué, sur un échantillon d'ovalbumine fraîchement préparé et recristallisé sept fois, quatre séries d'essais de solubilité à des activités variées d'ions hydrogène ( $\text{p}_{\text{aH}} = 4.84$  à  $5.22$ ). D'après les résultats de ces essais, on calcula  $a = \div 0.22$  et  $-\text{p}_{\text{aH}}$  étant  $= 4.90 - \beta = 5.41$ . De plus, on trouva que la façon dont  $\beta$  variait avec l'activité d'ions hydrogène pouvait, dans le domaine examiné, s'exprimer comme une fonction linéaire :

$$\beta = 5.41 + 1.7 (\text{p}_{\text{aH}} \div 4.90) \quad (2)$$

Ainsi donc, lorsque la solubilité d'un échantillon normal d'albumine d'œuf dans des solutions assez concentrées de sulfate d'ammonium est déterminée sous les conditions normales par nous choisies, la dépen-

dance entre la solubilité ( $s$ ), la teneur en sulfate d'ammonium ( $S$ ) et l'activité d'ions hydrogène ( $p_{\text{H}}$ ) peut s'exprimer par l'équation suivante:

$$\log s = -0.22 \cdot S + 5.41 + 1.7 (p_{\text{H}} \div 4.90) \quad (3)$$

A l'aide de cette équation ont été établies deux courbes étalons valables pour  $p_{\text{H}} = 4.90$ , où les valeurs de  $S$  sont portées en abscisses, les ordonnées étant représentées par les valeurs correspondantes de  $\log s$  et de  $s$  respectivement; dans le premier cas la courbe formait évidemment une ligne droite. Ces courbes furent utilisées comme courbes étalons en vue de résoudre la question de savoir si la solubilité d'un échantillon d'ovalbumine soumis à l'examen était normale ou non. Voici la manière dont nous avons opéré:

Les déterminations des solubilités furent effectuées sous les circonstances normales, et l'on s'efforçait de réaliser dans toutes les liqueurs filtrées une concentration en ions hydrogène aussi voisine de  $p_{\text{H}} = 4.90$  que possible. Pour les écarts d'avec ce chiffre, on introduisait, d'après l'équation (2), une correction, en ajoutant à  $\log s$  trouvé expérimentalement un terme:  $\div 1.7 (p_{\text{H}} \div 4.90)$ , qui faisait obtenir la valeur corrigée de  $\log s$  et, partant, la valeur corrigée de  $s$ . Ces valeurs corrigées correspondent à la solubilité qu'on aurait trouvée, si le  $p_{\text{H}}$  des liqueurs filtrées avait été exactement de 4.90. Les valeurs corrigées peuvent donc être directement utilisées pour la comparaison avec la solubilité de l'ovalbumine normale. Afin de faciliter cette comparaison, nous reproduisons graphiquement ces valeurs en même temps que les courbes étalons.

Ainsi que nous l'avons déjà signalé (p. 11) et qu'on le verra par les résultats numériques rassemblés dans la partie expérimentale, les corrections dont il s'agit lorsqu'on rapporte à  $p_{\text{H}} = 4.90$ , sont d'une importance essentielle, même quand  $p_{\text{H}}$  ne diffère que légèrement de 4.90. Pour cette raison nous avons mis en considération s'il ne serait pas opportun de pratiquer ces déterminations comparatives de solubilité à une activité ionique représentée par  $p_{\text{H}} =$  environ 4.6, vu qu'en ce cas une erreur faite dans le mesurage de la concentration ionique serait d'une importance moindre<sup>1)</sup>. Si néanmoins nous avons préféré effectuer les

<sup>1)</sup> Voir nos «Études sur les Protéines» IV, Figure 26. Compt. rend. Laboratoire Carlsberg, **12**, 245 (1917).

essais à  $p_{aH} = 4.90$  env., c'est parce que dans cette région ionique, la correction, comme on l'a vu dans ce qui précède, est proportionnelle à la différence ( $p_{aH} \div 4.90$ ), ce qui n'est pas le cas quand  $p_{aH} = 4.6$  env.

Les essais rapportés en détail dans la partie expérimentale et à l'occasion desquels on a mis en parallèle la solubilité de l'ovalbumine normale et celle d'ovalbumines soumises à des traitements variés, font voir ce qui suit:

1°. Une ovalbumine purifiée de la manière habituelle par quatre cristallisations, a pu être séparée en fractions ayant toutes la même teneur en phosphore et qui malgré cela ont accusé des différences de solubilité assez nettes bien que pas très grandes. La solubilité normale ne s'est rencontrée que pour la fraction la plus grande, celle cristallisée en premier lieu; au contraire, celle fournie par les liqueurs filtrées présentait une solubilité un peu supérieure à la normale, et, enfin, une troisième fraction, assez petite, offrait une solubilité très nettement supérieure à la normale (Section B. a).

Nous estimons qu'il ne s'agit pas ici d'impuretés qu'on n'aurait pas enlevées en préparant et purifiant l'albumine; à notre avis, on est en présence d'un réel fractionnement.

2°. A l'électrodialyse de solutions d'ovalbumine fraîchement préparées — opération qui, comme nous l'avons signalé à la p. 9, a pour conséquence un fractionnement du phosphore —, l'albumine riche en phosphore de la liqueur anodique et celle pauvre en phosphore de la liqueur intérieure, ont une solubilité à très peu près normale, tant que la teneur en phosphore ne s'écarte pas trop de la quantité ordinaire (7<sup>mg.</sup>5 de phosphore par g d'azote d'albumine). Dans une électrodialyse prolongée pendant assez longtemps et qui donne non seulement une liqueur intérieure contenant de l'albumine très pauvre en phosphore, mais finalement aussi une liqueur anodique dont l'albumine est relativement pauvre en phosphore, les fractions obtenues possèdent cependant une solubilité notablement supérieure à la normale, et d'autant plus grande que la teneur en phosphore est moindre. Il en est de même pour les fractions fournies par l'électrodialyse d'anciens échantillons d'ovalbumine; on devait bien s'y attendre puisque ces échantillons (v. plus loin) offrent déjà avant l'électrodialyse une solubilité dépassant la normale (Section B. d).

3°. Les échantillons provenant d'ovalbumine abandonnée durant

des années en présence de toluène dans la glace dans une glacière, présentent toujours une solubilité sensiblement plus grande que la normale, et, en ce qui concerne les vieilles solutions d'ovalbumine dialysées, la solubilité semble même d'autant plus grande que la teneur en phosphore est plus petite. Parmi un certain nombre de vieux échantillons d'ovalbumine conservés à l'état cristallin et entourés de l'eau-mère renfermant du sulfate d'ammonium, nous n'en avons examiné qu'un seul, lequel, contrairement aux autres, avait une teneur normale en phosphore et accusa néanmoins une solubilité supérieure à la normale (Section B. e.).

Comme conclusion principale de cette deuxième catégorie de nos recherches, nous pensons pouvoir formuler cet énoncé:

La solubilité de l'ovalbumine cristallisée dans des solutions assez concentrées de sulfate d'ammonium doit, dans des conditions données et pour des échantillons fraîchement préparés et purifiés de la manière usuelle, être considérée comme constante.

En somme, il ne semble pas exister de relation bien nette entre la teneur en phosphore d'une ovalbumine et sa solubilité, attendu qu'il a été possible d'en préparer des échantillons accusant, avec la teneur ordinaire en phosphore, une solubilité supérieure à la normale. D'un autre côté, il convient de faire ressortir que tous les échantillons d'ovalbumine préparés par nous et ayant une teneur en phosphore inférieure à environ 6<sup>mg</sup> de phosphore par g d'azote, possédaient une solubilité dépassant la normale et, d'une façon générale, d'autant plus que la teneur en phosphore était plus petite. Ce fait s'explique facilement, si la supposition émise précédemment (p. 8) est juste, c'est-à-dire si la molécule d'ovalbumine ne contient normalement qu'env. 5<sup>mg</sup>.8 de phosphore par g d'azote d'albumine.

## **Partie expérimentale.**

### *A. Méthodes d'analyse.*

#### a. Déterminations de phosphore.

1°. Dosage de la quantité de «Phosphore total». Après addition de 4 cm<sup>3</sup> d'acide sulfurique concentré, la quantité de solution protéique mesurée ou pesée fut desséchée dans un séchoir



à 100° C. environ, après quoi l'on procéda à l'oxydation et au traitement ultérieur décrit précédemment<sup>1)</sup>.

2°. Dosage du «Phosphore coagulable». La quantité mesurée ou pesée de la solution protéique fut soumise à la coagulation de la manière usuelle, après addition de 10 cm<sup>3</sup> d'acétate de sodium normal + 10 cm<sup>3</sup> d'acide acétique norm. + 10 cm<sup>3</sup> d'une solution saturée de sulfates sodique et potassique, avec de l'eau jusqu'au volume de 100 cm<sup>3</sup>. Le précipité, après séparation par filtration, fut lavé et traité comme décrit précédemment (*loc. cit.*).

3°. Dosage du «Phosphore précipitable par l'alcool». A la quantité mesurée ou pesée de la solution protéique on ajouta 5 cm<sup>3</sup> d'un mélange à volumes égaux d'acétate sodique normal, d'acide acétique norm. et de solution saturée sodique et potassique, après quoi la solution fut précipitée à la température ordinaire par addition d'alcool à 95 %, en agitant vivement. Si nous désignons par  $a$  cm<sup>3</sup> le volume total de la solution protéique et des 5 cm<sup>3</sup> de mélange, on précipita par, en tout,  $3 a$  cm<sup>3</sup> d'alcool lorsqu'il s'agissait de solutions d'albumine d'œuf, et par  $4 a$  cm<sup>3</sup> d'alcool dans le cas des protéines de sérum. Après abandon jusqu'au lendemain, avec agitation, on chauffa au bain d'eau bouillante, et le liquide fut maintenu pendant cinq minutes à l'ébullition ou du moins près de cette température. Ensuite on filtra, puis lava le précipité soigneusement à l'alcool chaud, à l'eau chaude, et de nouveau à l'alcool et à l'eau chauds. Enfin, on soumit le précipité à exactement le même traitement que celui indiqué pour le dosage du «phosphore coagulable».

#### b. Détermination de la Solubilité de l'Ovalbumine.

1°. Mode opératoire. Si l'on veut comparer les solubilités de différents échantillons d'albumine d'œuf dans des solutions concentrées de sulfate d'ammonium, il convient absolument — comme il a été développé dans l'introduction à ce mémoire (v. p. 10) — de prendre en considération tous les facteurs susceptibles d'exercer une influence sur l'état d'équilibre existant entre l'ovalbumine cristallisée et l'eau-mère qui l'entoure. Aussi, il faut toujours exécuter la détermination de solubilité suivant

<sup>1)</sup> Margrethe Sørensen: Compt. rend. Laboratoire Carlsberg, **15**, N° 10, (1925).

exactement le même procédé et, autant que possible, aux mêmes concentrations des réactifs. L'expérience a démontré que celui exposé ci-dessous, avec les concentrations indiquées, est bien approprié à la détermination de la solubilité de l'ovalbumine.

Avec la solution d'ovalbumine donnée, dont il faut connaître la teneur en N d'albumine et N d'ammoniaque par 100 cm<sup>3</sup>, on prépare une solution contenant par 100 cm<sup>3</sup> 0<sup>g</sup>.700 de N d'albumine et 1<sup>g</sup>.400 de N d'ammoniaque et dont la concentration en ions hydrogène correspond à peu près à  $p_{aH} = 4.90$ . Pour la préparation de cette solution on emploie, comme on le verra par l'exemple cité plus bas, en plus de la solution primitive d'albumine, une solution de sulfate d'ammonium (A) qui renferme, par centimètre cube, 100 mg de N d'ammoniaque, de l'eau et la quantité — déterminée par des mesurages préalables — d'ammoniaque norm. au 1/100 ou d'acide sulfurique norm. au 1/100 nécessaire pour donner à la solution un  $p_{aH} = 4.90$ .

La solution ainsi préparée est mise dans un verre cylindrique, pour y subir la cristallisation par addition ultérieure d'A, jusqu'à ce que le précipité amorphe ainsi formé ne se dissolve que difficilement par agitation; addition d'une goutte de matière amorçante et addition subséquente, en une dizaine de minutes, de petites doses d'eau, avec remuages répétés jusqu'à ce que l'on voie ce former une cristallisation bien nette. Ensuite, on ajoute dans le courant de deux jours et à quelques heures d'intervalle, en remuant bien, de faibles doses d'A, jusqu'à ce que le volume total d'eau et d'A ajoutés s'élève à 100 cm<sup>3</sup> par 100 cm<sup>3</sup> du volume primitif. Il va sans dire qu'il faut noter combien on aura ajouté, en tout, d'eau et d'A; il sera convenable d'employer, par 100 cm<sup>3</sup> de solution primitive, 5 cm<sup>3</sup> d'eau et 95 cm<sup>3</sup> d'A. Le mélange de cristaux et d'eau-mère aura alors la composition suivante:

100 cm <sup>3</sup> de solution primitive			≈ 0 <sup>g</sup> .7 de N d'alb. et 1 <sup>g</sup> .4 de N d'amm.
5	- d'eau		≈
95	- d'A		≈
			9 <sup>g</sup> .5 - - —
200 cm <sup>3</sup> de mélange			≈ 0 <sup>g</sup> .7 de N d'alb. et 10 <sup>g</sup> .9 de N d'amm.

Les additions terminées, le mélange de cristaux et d'eau-mère, avec 1 cm<sup>3</sup> de toluène, sera versé dans une bouteille, que l'on abandonne bouchée, pendant quelques heures, en agitant à plusieurs reprises, dans une étuve à eau à 18°, après quoi aura

lieu la distribution pour les essais particuliers de solubilité. Dans quelques bouteilles, soit 5, également installées dans l'étuve à 18°, on introduit à l'aide d'une pipette des volumes égaux, soit 100 cm<sup>3</sup>, du mélange de cristaux et d'eau-mère, en agitant celui-ci énergiquement avant chaque mesure. Ensuite, afin d'établir l'équilibre de solubilité, on ajoute — lentement et en agitant bien —, par 100 cm<sup>3</sup> de mélange de cristaux et d'eau-mère, 20 cm<sup>3</sup> de l'un des mélanges ci-après, qui doivent avoir d'avance la température de 18°:

1°	100 cm <sup>3</sup>	d'eau	+	0 cm <sup>3</sup>	d'A
2°	90	-	-	+ 10	-
3°	80	-	-	+ 20	-
4°	70	-	-	+ 30	-
5°	60	-	-	+ 40	-

Dans chacun des mélanges ainsi préparés, il y aura alors, par 100 cm<sup>3</sup>, 0g.292 de N d'albumine, tandis que la teneur en N d'ammoniaque variera de 4g.542 à 5g.208<sup>1)</sup>.

Lorsque cette addition aura eu lieu, on bouche les bouteilles avec des bouchons de liège bien nettoyés par de l'eau bouillante, et on les abandonne dans l'étuve à 18°, en les secouant plusieurs fois par jour afin d'atteindre l'équilibre de solubilité. La moitié du mélange est filtrée après quatre jours d'abandon et le reste après six jours, et si l'état d'équilibre a été réalisé — ce qui est presque toujours le cas, — la teneur en albumine du premier et du second filtrat devra être la même, dans les limites des erreurs expérimentales.

Les filtrations s'effectuent dans une chambre dont la température est aussi voisine de 18° que possible. Les mesures de

<sup>1)</sup> Exemple: Une de nos solutions d'ovalbumine contenait, par 100 cm<sup>3</sup>, 2g.909 de N d'albumine et 2g.866 de N d'ammoniaque.

On mélangea

72.2 cm<sup>3</sup> de solution d'alb. ∞ 2g.100 de N d'alb. et 2g.069 de N d'ammon.

21.3 - d'A ∞ — 2g.130 - - —

79.8 - de H<sub>2</sub>SO<sub>4</sub> n/100

126.7 - d'eau

300 cm<sup>3</sup> ∞ 2g.100 de N d'alb. et 4g.199 de N d'ammon.

donc par 100 cm<sup>3</sup>: 0g.700 de N d'alb. et 1g.400 de N d'ammon.

Les 300 cm<sup>3</sup> furent précipités avec, en tout, 285 cm<sup>3</sup> d'A et 15 cm<sup>3</sup> d'eau, et des 600 cm<sup>3</sup> de mélange de cristaux et d'eau-mère on enleva 5 × 100 cm<sup>3</sup> pour les soumettre à des essais de solubilité.

précaution à observer ont été indiquées antérieurement<sup>1)</sup> (Entonnoirs au bord émoulu, couvert par une plaque de verre également émoulu, etc., rejet de la portion de 10 à 20 cm<sup>3</sup> qui a passé la première). Dans des portions pesées des liqueurs filtrées, on fait le dosage, à la manière usuelle, de N d'albumine et de N ammoniacal, de même qu'on y mesure la concentration en ions hydrogène.

Dans le procédé que nous venons d'exposer, procédé où les doses ajoutées sont mesurées au lieu d'être pesées, et où dans les calculs on ne tient pas compte des modifications de volume susceptibles de se produire par suite du mélange des différentes solutions, les mélanges obtenus n'acquièrent évidemment pas exactement les concentrations désirées; pourtant, le procédé est suffisamment précis pour assurer dans tous les essais la même concentration protéique initiale; l'N d'albumine et l'N d'ammoniaque aussi bien que  $p_{aH}$  sont déterminés avec précision dans chaque filtrat. Si la valeur de  $p_{aH}$  s'écarte de 4.90, on applique une correction — voir plus loin — relative à la solubilité et correspondant à l'écart.

2°. Courbes étalons. Dans la supposition que la solubilité de l'ovalbumine normale dans les solutions concentrées de sulfate d'ammonium, sous des conditions données, varie toujours de la même manière, cette dépendance peut être représentée par des courbes convenablement choisies. Nous employons deux courbes étalons, où les abscisses représentent la concentration en sulfate d'ammonium, exprimée en grammes de ce sulfate (S) par 100 g d'eau, tandis que les ordonnées indiquent la solubilité de l'hydrate d'œuf exprimée en grammes de cet hydrate (s) par 100 g d'eau pour l'une des deux courbes, et par le logarithme de cette quantité ( $\log s$ ) pour l'autre. En exécutant les expériences sur lesquelles sont basées ces courbes étalons, nous nous sommes astreints à suivre bien exactement le procédé décrit plus haut, de telle sorte que les courbes ne sont valables que pour les circonstances indiquées (équilibre de solubilité et non de cristallisation, concentration initiale en albumine, etc.). Comme valeur étalon de  $p_{aH}$ , nous avons choisi 4.90, et puisque la grande influence exercée sur la solubilité par l'activité des ions hydrogène de l'eau-mère rend strictement nécessaire d'apporter des correc-

<sup>1)</sup> Compt. rend. Laboratoire Carlsberg, 12, 170 (1927).

tions correspondantes même à de faibles écarts d'avec cette valeur d'étalon, il a fallu, en traçant les courbes étalons, porter notre attention toute particulière sur ces écarts. Voici notre mode d'opérer:

Sur un échantillon d'albumine d'œuf fraîchement préparé et soumis à sept recristallisations successives, on a effectué quatre séries de déterminations de solubilité, en réglant l'activité des ions hydrogène de façon à la faire correspondre dans les quatre séries à  $p_{aH} = 4.84, 4.92, 4.98$  et  $5.22$ , respectivement.

Les résultats de ces essais sont rassemblés dans le Tableau 1, où S est la marque que portait l'échantillon dont il s'agit.

Reproduits graphiquement suivant le principe proposé par Edwin J. Cohn (v. p. 13), avec emploi des valeurs de S et de  $\log s$  respectivement comme abscisses et ordonnées, ces résultats expérimentaux ont pu être représentés par quatre lignes droites à très peu près parallèles, correspondant à la formule:

$$\log s = \alpha \cdot S + \beta.$$

Pour chacune des quatre lignes,  $\alpha$  avait la valeur de  $\div 0.22$ , tandis que celle de  $\beta$ , différente dans les quatre séries, était d'autant plus grande que  $p_{aH}$  était plus grand. En outre, il fut constaté que la variation subie par  $\beta$  par suite de la variation de l'activité des ions hydrogène, dans le domaine examiné, pouvait s'exprimer comme une fonction linéaire, et de telle manière qu'une augmentation de 0.1 dans la valeur de  $p_{aH}$  correspondait à une augmentation de 0.17 dans la valeur de  $\beta$ .

Or, de la cinquième colonne verticale du Tableau 1, il ressort qu'aucune des liqueurs filtrées n'avait exactement l'activité d'ions hydrogène à laquelle on avait visé en faisant le plan de l'essai. Pourtant, en tirant parti de la dépendance signalée plus haut entre les valeurs de  $\beta$  et de  $p_{aH}$ , il est facile de corriger  $\beta$  et partant  $\log s$  relativement à la faible différence constatée entre le  $p_{aH}$  trouvé et celui voulu. La huitième colonne verticale du tableau contient les valeurs ainsi corrigées de  $\log s$ ; pour l'essai N° 1, par exemple, où  $p_{aH}$  est trouvé de 0.001 plus bas que le chiffre voulu, 4.98, la correction sera positive:

$$\div 0.192 + 0.17 \cdot 0.01 = \div 0.190.$$

A l'aide des valeurs ainsi corrigées de  $\log s$  — et qui correspondent à la solubilité que, dans des conditions égales d'ailleurs,

Tableau I.

Mrg.	No	Pour 100 g		Activité d'ions hy- drique du filtrat (Seconde filtration)	log s	log s corr.	$\alpha \cdot S$	$\beta =$ $\log s(\text{corr.}) \div \alpha \cdot S$	$\beta$ moy- enne	log s rapporté à $pH = 4.90$		s (corr.) ( $pH = 4.90$ )
		Hydrate d'ouf s	Sulfate d'ammon. s		log s rapporté à $pH$					Corr. $-1.7 (pH - 4.90)$	log s (4.90)	
SI ○	1	0.643	26.120	4.979	-0.192	-0.190	-5.746	5.556	5.561	-0.134	-0.326	0.472
	2	0.233	28.192	4.982	do.	-0.636	-6.202	5.566		-0.139	-0.772	0.169
	3	1.714	25.807	5.200	0.234	0.268	-5.678	5.946		-0.510	-0.276	0.529
	4	1.116	26.805	5.219	0.048	0.050	-5.897	5.947		-0.532	-0.484	0.328
	5	0.687	27.946	5.240	-0.163	-0.197	-6.148	5.951		-0.578	-0.741	0.181
	6	0.422	29.000	5.229	-0.375	-0.390	-6.380	(5.990)		-0.559	-0.934	0.116
	7	0.246	29.895	5.232	-0.609	-0.629	-6.577	5.948		-0.564	-1.173	0.067
SII △	8	0.681	24.969	4.831	-0.167	-0.152	-5.493	5.341	5.307	0.117	-0.050	0.891
	9	0.397	26.042	4.838	-0.401	-0.398	-5.729	5.331		0.105	-0.296	0.506
	10	0.251	27.012	4.847	-0.600	-0.612	-5.943	5.331		0.090	-0.510	0.309
	11	0.136	28.006	4.859	-0.866	-0.898	-6.161	5.263		0.070	-0.796	0.160
	12	0.079	29.167	4.867	-1.102	-1.148	-6.417	5.269		0.056	-1.046	0.090
SIH □	13	0.916	24.773	4.916	0.038	-0.031	-5.450	5.419	5.426	-0.027	-0.065	0.861
	14	0.625	25.594	4.921	-0.204	-0.206	-5.631	5.425		-0.036	-0.240	0.575
	15	0.349	26.788	4.931	-0.457	-0.476	-5.893	5.417		-0.053	-0.510	0.309
	16	0.207	27.787	4.913	-0.684	-0.672	-6.113	5.441		-0.022	-0.706	0.197
SIIV ○	17	0.125	28.905	4.956 (inertant)	0.993	-0.964	-6.339	(5.395)		-0.095	-0.998	0.100

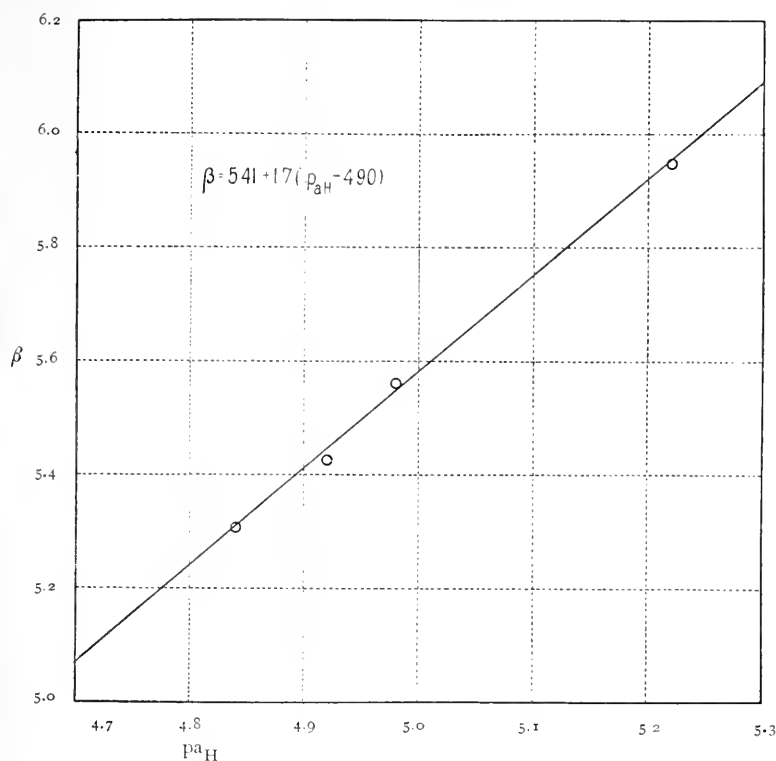


Fig. 1.

on aurait trouvée, si l'activité des ions hydrogène eût été précisément celle qu'on avait tâché d'obtenir —, on est maintenant à même, pour chaque essai en particulier, de calculer  $\beta$  au moyen de la formule:

$$\beta = \log s \text{ (corr.) } \div a \cdot S,$$

où  $a$  est  $\div 0.22$  et  $S$  a la valeur trouvée directement par l'essai de solubilité. Les valeurs ainsi calculées de  $\beta$ , ainsi que leurs valeurs moyennes, sont inscrites aux dixième et onzième colonnes du tableau.

La Figure 1 est une représentation graphique de la dépendance existant entre les quatre valeurs moyennes trouvées pour  $\beta$  et les valeurs correspondantes de  $p_{aH}$ .

Il résulte de la figure que la dépendance entre  $\beta$  et  $p_{aH}$  peut s'exprimer comme une fonction linéaire de la forme suivante:

$$\beta = 5.41 + 1.7(p_{aH} \div 4.90).$$

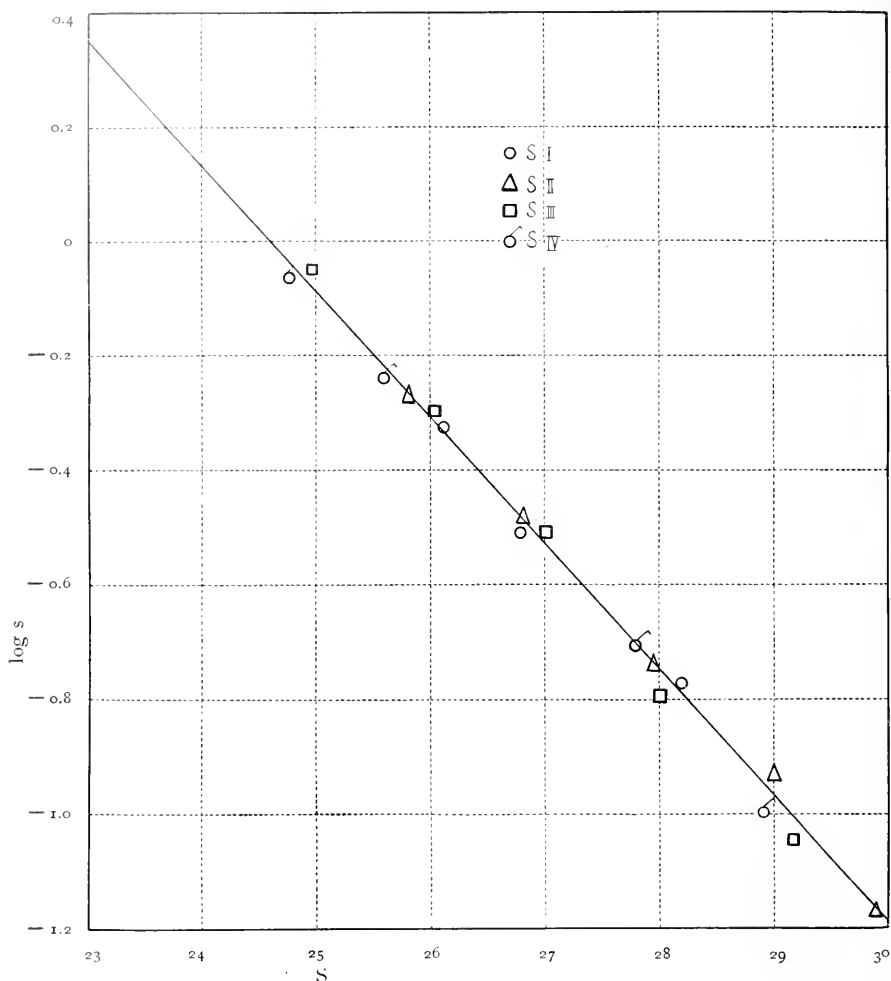


Fig. 2.

Par conséquent, la solubilité de l'ovalbumine dans les conditions normales se laisse exprimer par la fonction linéaire que voici :

$$\log s = \div 0.22 \cdot S + 5.41 + 1.7 (\text{pa}_H \div 4.90).$$

C'est sur la base de cette équation que, comme il a été déjà mentionné dans le premier chapitre (p. 14), on a dressé les deux courbes étalons, se rapportant l'une et l'autre à  $\text{pa}_H = 4.90$ ; comme abscisses de ces courbes on a utilisé des valeurs convenablement choisies de  $S$ , et comme ordonnées les valeurs  $y$  correspondantes, calculées au moyen de l'équation, pour  $\log s$



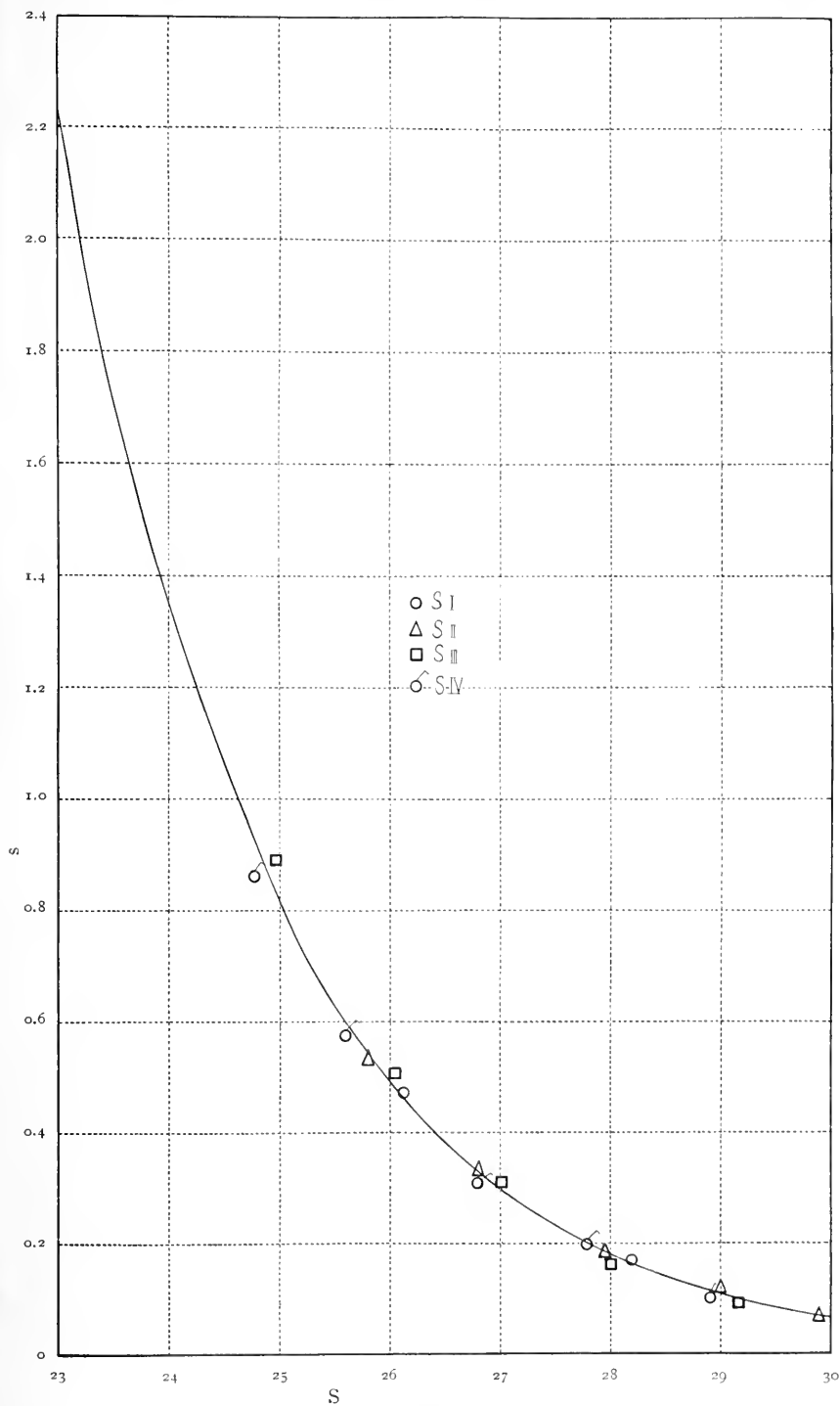


Fig. 3.

et  $s$  respectivement. Sur les figures 2 et 3, la ligne droite et la courbe représentent, respectivement, les courbes étalons construites sur la base de l'équation.

Dans tous les essais de solubilité rapportés dans la suite, nous avons fait usage de ces courbes étalons comme courbes de comparaison, en effectuant la détermination de solubilité à une activité d'ions hydrogène correspondant autant que possible à  $p_{\text{H}} = 4.90$ , et en corrigeant — ainsi qu'il a été mentionné dans l'introduction (p. 14) — relativement à la différence entre le  $p_{\text{H}}$  trouvé et 4.90.

Les essais rassemblés au Tableau I peuvent naturellement, eux aussi, être rapportés à  $p_{\text{H}} = 4.90$ , en ajoutant à la valeur trouvée expérimentalement pour  $\log s$ , le terme de correction  $\div 1.7$  ( $p_{\text{H}} \div 4.90$ ). Ce rapport à  $p_{\text{H}} = 4.90$  a été fait dans les trois dernières colonnes verticales du Tableau I, et les valeurs corrigées ainsi calculées pour  $\log s$  et pour  $s$  se trouvent insérées sur les courbes étalons (Fig. 2 et 3). Les courbes étant construites sur la base de ces essais, les valeurs corrigées devront naturellement se grouper autour des courbes étalons; si malgré cela on les a rapporté à  $p_{\text{H}} = 4.90$ , c'est pour donner un exemple de notre procédé, d'un côté, et, de l'autre, dans l'intention de faire voir la concordance qui existe entre chaque essai des quatre séries et les courbes calculées sur les valeurs moyennes.

### *B. Échantillons d'ovalbumine examinés.*

#### *a. Cristallisation fractionnée.*

En préparant l'échantillon d'albumine d'œuf servant à ces expériences, on a évalué le rapport entre la teneur en phosphore et celle en azote non seulement dans la matière première purifiée, mais aussi dans les liqueurs filtrées obtenues soit par la recristallisation de l'albumine, soit par la reprécipitation de la globuline.

1°. Globuline d'œuf. Le blanc d'œuf bien fouetté fut précipité au moyen d'un volume égal d'une solution saturée de sulfate d'ammonium, et le précipité séparé par filtration fut lavé avec une solution à demi saturée de sulfate d'ammonium. Ensuite, ayant enlevé le précipité au filtre, on le délaya dans un volume d'eau connu, puis, après mesure du volume total, on sépara par filtration les matières visqueuses restées indissoutes. On précipita le filtrat par une solution saturée de sulfate d'ammonium

en quantité telle qu'on obtint de nouveau une solution à demi saturée, après quoi on sépara par filtration la globuline précipitée; dans la liqueur filtrée, qui fut désignée par  $G_I$ , on dosa l'«azote coagulable» et le «phosphore coagulable». La reprécipitation fut effectuée, en tout, cinq fois; cependant les deux derniers filtrats se sont trouvés ne renfermer qu'une quantité infime de protéine coagulable, et nous n'en avons donc pas fait d'analyse. Dans les trois autres filtrats, la teneur en phosphore, exprimée en mg de phosphore par g d'azote, s'est chiffré comme suit:

$G_I$ .....	5.3
$G_{II}$ .....	3.6
$G_{III}$ .....	1.7

La globuline cinq fois reprécipitée, soumise à une dialyse, se partagea en une fraction soluble dans l'eau et une autre qui ne s'y dissolvait pas; la teneur en phosphore s'est chiffrée ainsi:

Fraction soluble .....	1.9
Fraction insoluble .....	1.8

La globuline d'œuf contient donc, comme on l'a déjà vu dans l'introduction (p. 2), une proportion de phosphore de beaucoup inférieure à celle de l'albumine d'œuf.

2°. Albumine d'œuf. De la liqueur filtrée provenant de la globuline précipitée en premier lieu, on fit cristalliser l'ovalbumine, qu'on sépara par filtration, puis qu'on lava et fit recristalliser trois fois à la manière habituelle. La teneur en phosphore — exprimée en mg de phosphore coagulable par g d'azote coagulable — des filtrats obtenus par les trois recristallisations fut évaluée, respectivement, à 6.6, 6.9 et 6.8.

L'ovalbumine cristallisée par quatre fois et qui contenait 7<sup>mg</sup>.64 de phosphore coagulable par g d'azote coagulable, servit de matière première pour les essais de fractionnement, lesquels furent pratiqués à trois différentes activités d'ions hydrogène:

Essai A: $p_{aH}$ =	5.0
- B: - =	4.5
- C: - =	5.5

Après addition des doses respectivement d'ammoniaque norm. au  $1/10$  ou d'acide sulfurique norm. au  $1/10$  évaluées par des essais préalables et nécessaires pour donner aux solutions l'activité voulue d'ions hydrogène, on ajouta dans chacun des trois essais

de fractionnement une quantité de sulfate d'ammonium suffisante pour faire cristalliser dans l'espace de deux ou trois jours une notable partie d'albumine. Les précipités ainsi obtenus, désignés par  $A_I$ ,  $B_I$  et  $C_I$ , furent séparés par filtration, et dans les liqueurs filtrées on fit cristalliser encore de l'ovalbumine  $A_{II}$ ,  $B_{II}$  et  $C_{II}$  par addition ultérieure d'une solution saturée de sulfate d'ammonium. Additionnée d'encore une portion de solution saturée de sulfate d'ammonium, les filtrats de ces précipités ont fourni encore de petites quantités d'albumine, lesquelles — désignées par  $A_{III}$ ,  $B_{III}$  et  $C_{III}$  — présentaient comme toutes les autres fractions un bel aspect cristallin et étaient tout à fait exemptes d'impuretés amorphes.

Les diverses fractions contenaient les quantités suivantes de phosphore, exprimé en mg par g d'azote coagulable:

	I	II	III
A ( $p_{aH} = 5.0$ ).....	7.63....	7.58....	7.67
B ( - = 4.5).....	7.73....	7.78....	7.56
C ( - = 5.5).....	7.72....	7.52....	7.73

La moyenne de ces quantités est de 7.66, résultat qui s'accorde très bien avec la teneur en phosphore de la matière première (7.64). Les écarts avec la moyenne sont tous compris entre les limites des erreurs expérimentales. Dans cette cristallisation fractionnée, il ne s'est donc pas produit de fractionnement de la teneur en phosphore.

3°. Solubilité des fractions d'albumine. Sur les quantités d' $A_I$ ,  $B_I$  et  $C_I$  restant après l'exécution des analyses, on a effectué des déterminations de solubilité par le procédé décrit plus haut (p. 17). Il ne restait que des quantités peu considérables des autres fractions; pourtant, en mélangeant ensemble  $A_{II}$ ,  $B_{II}$  et  $C_{II}$ , on put obtenir des matériaux suffisants pour une série de cinq déterminations (marquées  $M_{II}$ ), tandis que le mélange d' $A_{III}$ ,  $B_{III}$  et  $C_{III}$  n'en fournit que pour une seule détermination (marquée  $M_{III}$ ).

Les résultats de ces déterminations de solubilité sont groupés dans le Tableau II, qui n'a pas besoin d'explication.

Dans les trois dernières colonnes verticales de ce tableau, les valeurs expérimentalement trouvées sont rapportées à  $p_{aH} = 4.90$ , et les valeurs ainsi calculées et corrigées de  $\log s$  et de  $s$ , sont insérées sur les courbes étalons, par forme de comparaison avec la solubilité normale (Fig. 4 et 5).

Tableau II.

Mrq.	No	Pour 100 g d'eau		Activité d'ions hy- drogène du filtrat $p_{aH}$	log s	log s rapporté à $p_{aH} = 4.90$		s (corr.) (4.90)
		Hy- drate d'œuf s g	Sulfate d'ammo- nium S g			Corr. $-1.7(p_{aH} - 4.90)$	log s (corr.) (4.90)	
A <sub>I</sub> ○	1	1.175	24.510	4.90	0.070	0	0.070	1.175
	2	0.720	25.500	4.93	-0.143	-0.051	-0.194	0.640
	3	0.470	26.513	4.93	-0.328	-0.051	-0.379	0.418
	4	0.276	27.555	4.97	-0.559	-0.119	-0.678	0.210
	5	0.167	28.395	4.95	-0.777	-0.085	-0.862	0.137
B <sub>I</sub> △	6	1.089	24.238	4.87	0.037	0.051	0.088	1.225
	7	0.674	25.361	4.90	-0.171	0	-0.171	0.675
	8	0.398	26.403	4.92	-0.400	-0.034	-0.434	0.368
	9	0.236	27.410	4.91	-0.627	-0.017	-0.644	0.227
C <sub>I</sub> □	10	0.661	25.749	4.94	-0.180	-0.068	-0.248	0.565
	11	0.242	27.746	4.96	-0.616	-0.102	-0.718	0.191
	12	0.420	26.712	4.94	-0.377	-0.068	-0.445	0.359
M <sub>II</sub> ⊗	13	1.512	24.214	4.90	0.180	0	0.180	1.512
	14	1.107	25.179	4.94	0.044	-0.068	-0.024	0.946
	15	0.618	26.227	4.91	-0.209	-0.017	-0.226	0.594
	16	0.377	27.299	4.93	-0.424	-0.051	-0.475	0.335
	17	0.243	28.487	4.93	-0.614	-0.051	-0.665	0.216
M <sub>III</sub> ⊗	18	0.551	27.066	4.90	-0.259	0	-0.259	0.551

Un coup d'œil sur les Fig. 4 et 5 fera voir que A<sub>I</sub>, B<sub>I</sub> et C<sub>I</sub> ont une solubilité tout à fait normale, vu que les écarts sont tous — sauf peut-être une seule exception — moindres que les erreurs d'expériences avec lesquelles il faut compter ici. Au contraire, M<sub>II</sub> et surtout M<sub>III</sub> possèdent, paraît-il, une solubilité supérieure à la normale. D'autre part, on voit par la Fig. 4 que la ligne droite qui peut être tirée par les cinq points représentant les essais faits avec M<sub>II</sub>, a un degré d'inclinaison légèrement différent de la normale.

De nos déterminations de solubilité il résulte donc qu'il s'est produit un fractionnement appréciable, bien que pas très marqué. Or, étant donné que — comme il a déjà été mentionné — la quantité de phosphore est la même dans toutes les fractions, il s'ensuit que la différence de solubilité tient probablement à ce que

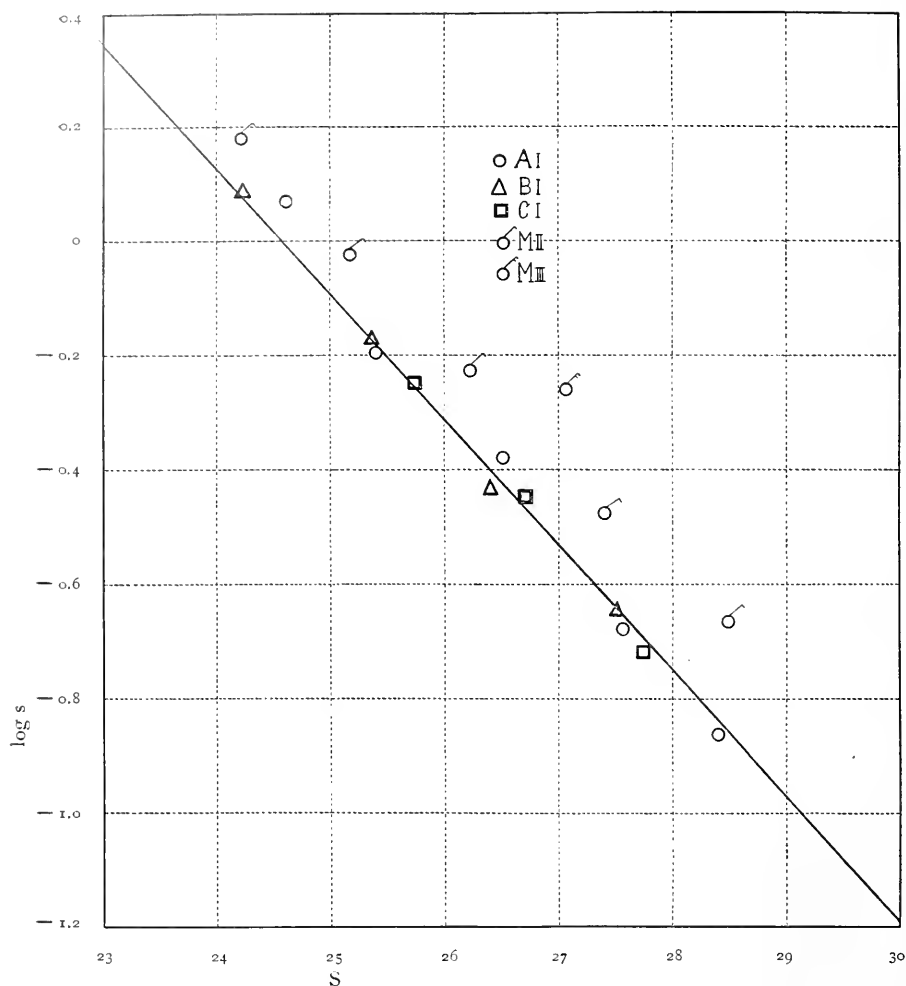


Fig. 4.

dans la cristallisation fractionnée quelques-uns des complexes non phosphorés de l'ovalbumine se seraient répartis inégalement entre les différentes fractions, et ce la évidemment de telle façon que la combinaison de complexes donnant le produit le plus difficilement soluble aurait cristallisé la première.

#### b. Traitement par l'ammoniaque.

Il est connu que la caséine en solution alcaline tant soit peu concentrée dégage tout ou partie de son phosphore sous forme

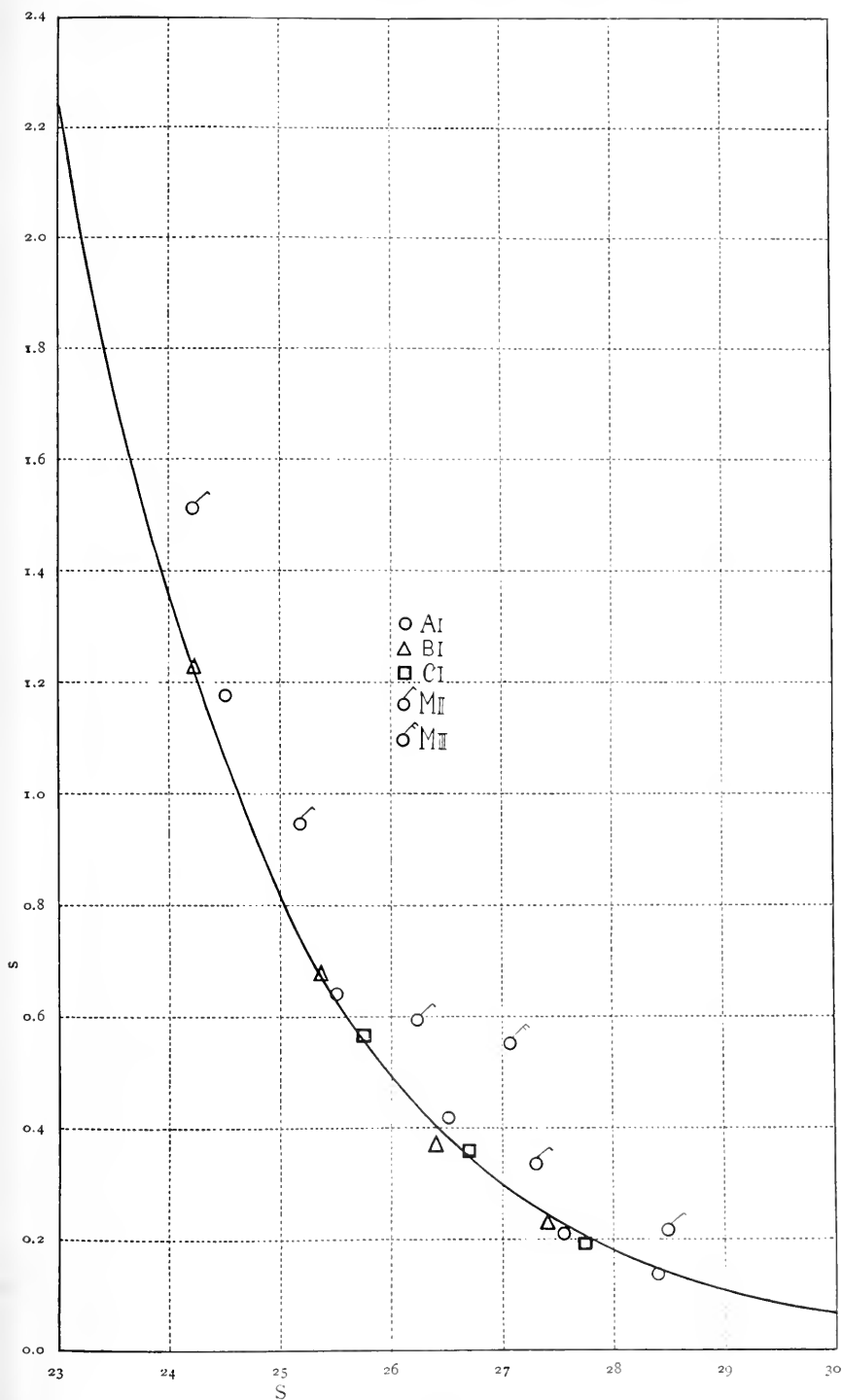


Fig. 5.

de phosphate. Or, on pourrait se figurer que l'ovalbumine soumise à l'action de quelque réactif alcalin arriverait à se défaire pareillement de son groupe phosphoré, et la question se pose alors de savoir s'il y aurait moyen d'opérer une telle scission de l'ovalbumine sans en modifier les propriétés caractéristiques, telles que sa faculté de cristallisation ou sa solubilité. Dans le but d'éclaircir cette question, nous avons fait un seul essai, que nous allons relater.

Il portait sur une albumine d'œuf fraîchement préparée, recristallisée six fois, puis dialysée (marquée E). Dans 100 cm<sup>3</sup> de solution, elle contenait 28.253 de N d'albumine et 17<sup>mg</sup>.70 de phosphore, donc 7<sup>mg</sup>.86 de phosphore par g de N d'albumine.

Par des essais préalables, on a apprécié combien d'ammoniaque normale on pourrait ajouter à des portions mesurées de cette solution d'ovalbumine sans que, abandonnée jusqu'au lendemain à la température ordinaire, elle perdît sa faculté de cristallisation. Sur la base des données ainsi obtenues, on procéda à l'exécution de l'expérience principale.

375 cm<sup>3</sup> de la solution d'ovalbumine furent mélangés avec 150 cm<sup>3</sup> d'ammoniaque normale; l'activité des ions hydrogène de la solution se trouva alors correspondre à  $p_{aH} = 9.7$  env. Après abandon jusqu'au lendemain, on ajouta en agitant 150 cm<sup>3</sup> d'acide sulfurique normal et en même temps 150 cm<sup>3</sup> d'une solution saturée de sulfate d'ammonium, destinée à favoriser la séparation à l'état floconneux de l'albumine dénaturée que la solution expérimentée pouvait renfermer. On obtint ainsi un précipité très minime, qui fut séparé par filtration, sur quoi l'on fit cristalliser l'ovalbumine de la manière habituelle. On sépara par filtration le précipité cristallisé, puis soumit à l'analyse la liqueur filtrée (Filtrat I), tandis que le précipité, après lavage à la manière ordinaire, fut dissous et soumis à une nouvelle cristallisation. Après séparation par filtration du précipité, la liqueur filtrée (Filtrat II) ainsi que la dissolution du précipité furent analysées.

Comme les filtrats contenaient de fortes quantités de sulfate d'ammonium et seulement de faibles doses d'ovalbumine, il s'ensuit que les analyses, et en particulier les dosages des quantités infimes de phosphore, ont été entachées de graves erreurs. On a trouvé dans

le Filtrat I: 7<sup>mg</sup>.47 de phosphore coag. par g de N d'albumine coag.

le Filtrat II: 8<sup>mg</sup>.36 - - - - -



La dissolution du précipité avait un volume de 300 cm<sup>3</sup> et contenait en tout 7<sup>g</sup>.245 de N d'albumine coagulable et 56<sup>mg</sup>.19 de phosphore coagulable, donc 7<sup>mg</sup>.76 de phosphore coag. par g de N d'albumine coag.

De ce résultat il ressort que la partie de beaucoup la plus grande de l'ovalbumine employée a été récupérée sous la forme cristalline et ayant une teneur parfaitement normale en phosphore.

### c. Traitement par l'alcool.

On a vu déjà dans l'introduction (v. p. 4) que l'albumine du sérum de cheval, par précipitation à froid par l'alcool, puis lavage à froid avec de l'alcool, abandonne la presque-totalité de son phosphore coagulable sans perdre sa faculté de cristallisation. Puisque dans les analyses d'ovalbumine pure nous n'avons jamais pu constater de différence entre la proportion de «phosphore coagulable» et celle de «phosphore précipitable par l'alcool» (v. p. 17), on pouvait à peine s'attendre à ce que l'alcool pût à froid extraire de l'ovalbumine des matières contenant du phosphore. Quoique cela, nous avons entrepris un essai à cet effet, essai que nous allons relater.

A 25 cm<sup>3</sup> de la solution susmentionnée d'ovalbumine dialysée E (v. p. 32), contenant en tout 563 mg de N d'albumine et 4<sup>mg</sup>.43 de phosphore, on ajouta env. 2 cm<sup>3</sup> d'ammoniaque normale, après quoi la solution accusa une réaction faiblement alcaline au papier de tournesol. Refroidie dans l'eau glacée, cette solution fut versée lentement et en agitant dans 200 cm<sup>3</sup> d'alcool absolu refroidi à  $\div 15^{\circ}$ , ce qui eut pour effet d'élever la température du mélange à  $\div 10^{\circ}$ . Comme il ne se produisit guère de dépôt, ni aussitôt ni après abandon durant toute la nuit à  $\div 7^{\circ}$ , on ajouta le lendemain 5 cm<sup>3</sup> d'un mélange refroidi de volumes égaux d'acétate de sodium normal, d'acide acétique norm. et d'une solution saturée de sulfate sodique et potassique, ce qui eut pour effet que le tout se figea en une gelée demi-transparente, laquelle abandonnée à froid jusqu'au lendemain et remuée à plusieurs reprises, fit tomber un dépôt volumineux que l'on put séparer par filtration. Celle-ci ainsi que le lavage du dépôt furent pratiqués à  $\div 7^{\circ}$ ; on lava deux fois avec de l'alcool à 95 %, trois fois à l'alcool absolu, enfin trois fois à l'éther pur. Cela fait, le dépôt fut soumis dans un appareil de Soxhlet à l'extraction

au moyen de l'éther, et finalement débarrassé de ce dernier par abandon dans le vide sulfurique.

Après avoir fait évaporer l'alcool et l'éther de tous les extraits obtenus, on dosa dans le résidu le N ammoniacal, N total et phosphore total. En dehors de l'azote ammoniacal on ne put déceler que des doses infimes d'azote, dont la totalité n'atteignait pas 1 mg; l'ensemble du phosphore total se chiffrait par 0<sup>mg</sup>.036, quantité inférieure à 1 % du taux total de phosphore dans l'ovalbumine employée pour cette expérience.

L'ovalbumine sèche délivrée de l'éther et toujours mélangée des sels ajoutés, était insoluble dans l'eau et tout à fait dénaturée; sa teneur en phosphore total était de 7<sup>mg</sup>.56 par g de N total, donc parfaitement normale.

De ce qui précède, il résulte que même dans les conditions choisies nous ne sommes pas parvenus à extraire des matières phosphorées de l'ovalbumine, ce qui rend vraisemblable que les complexes phosphorés font partie intégrante des molécules d'ovalbumine.

#### d. Électrodialyse.

1<sup>o</sup> Mode opératoire. Pour ces recherches on a fait usage de solutions d'albumine d'œuf bien dialysées, très pauvres en électrolytes et qui furent soumises à l'électrodialyse dans l'appareil de Pauli ordinaire<sup>1</sup>). Nous nous sommes servis de deux membranes de collodion, entre lesquelles était placée la solution d'ovalbumine, la liqueur intérieure, tandis que les liqueurs extérieures, tant du côté de l'anode que de la cathode, étaient constituées par de l'eau distillée bouillie. Lorsque les liquides eurent été introduits dans l'appareil, on l'installa dans la glacière et l'y laissa en repos pendant deux à trois jours sans le faire traverser par un courant, de façon à pouvoir s'assurer qu'en l'absence de celui-ci les membranes étaient impénétrables à l'ovalbumine. Une fois ceci constaté, on fit arriver un courant de la ligne de transmission de la lumière (220 volts), en insérant dans cette ligne une résistance si forte que l'intensité du courant fut inférieure à 100 milliampères. D'ordinaire, c'est seulement aux stades initiaux de l'électrodialyse qu'il fallait insérer une résistance; dans la suite, lorsque les électrolytes contenus dans la liqueur intérieure eurent

<sup>1</sup>/ Biochem. Zeitschrift, **152**, 357 (1924).

disparu presque complètement, l'intensité du courant, en l'absence d'une résistance, se réduisit à 30 à 20 milliampères.

Aussitôt le courant fermé, l'ovalbumine commença de pénétrer par la membrane de collodion dans le compartiment de l'anode, tandis que celui de la cathode demeura exempt d'albumine pendant des journées. Lorsque, le courant coupé, puis les liqueurs anodique et cathodique évacuées, les deux compartiments bien lavés avec de l'eau eurent été remplis d'eau pure, on vit encore de très faibles doses pénétrer dans le compartiment anodique; toutefois, la pénétrabilité cessa tout à fait du jour au lendemain. Quand la direction du courant fut changée et que, par conséquent, le compartiment cathodique devint anodique, l'albumine passa à travers la membrane correspondante, alors que l'autre membrane, qui était auparavant pénétrable, se montra dès lors complètement impenétrable à l'albumine. Quoique nous ayons fait plusieurs essais en vue d'éclaircir ces faits, nous ne sommes pas encore à même de donner une explication de tous points satisfaisante de ce singulier phénomène.

Dans le cas où l'activité des ions hydrogène de la solution d'albumine correspondait, par exemple, à  $p_{aH} = 4.9$ , les liqueurs anodiques présentaient un  $p_{aH}$  plus bas, soit 4.6 à 4.7, alors que celui des liqueurs cathodiques était de beaucoup plus élevé, soit 6.5 à 7.0, et d'autant plus élevé que la teneur en électrolytes de la liqueur intérieure était plus grande; l'activité des ions hydrogène de la liqueur intérieure ne se modifiait guère au cours de l'électrodialyse.

Après avoir fait durer l'électrodialyse un temps convenable — ordinairement un jour —, on soutira les liqueurs anodique et cathodique pour les remplacer par de l'eau pure, après quoi l'électrodialyse fut continuée. La liqueur intérieure, qui ne fut soustraite qu'à la clôture de l'électrodialyse, se montrait d'ordinaire un peu louche par suite d'un peu de dépôt en suspension, que l'on put facilement séparer par filtration et qui était vraisemblablement formé d'ovalbumine dénaturée.

Sur toutes les solutions soutirées on préleva des échantillons pour le dosage de N total et du phosphore total, parfois aussi en vue d'autres estimations, puis les solutions albumineuses furent précipitées au moyen d'un excès de solution saturée de sulfate d'ammonium. On sépara par filtration le dépôt précipité et le fit dissoudre dans l'eau, puis amena la dissolution à cristalliser de

la manière ordinaire, généralement avec succès lorsque pendant la dialyse l'intensité du courant n'avait pas dépassé le degré indiqué plus haut. Les précipités cristallisés furent séparés par filtration, puis lavés et recristallisés comme à l'ordinaire, au moins deux fois. Enfin, les solutions des albumines recristallisées — lesquelles n'étaient jamais incolores, mais offraient une teinte nettement jaunâtre ou brunâtre — furent soumises à l'analyse et utilisées à des déterminations de solubilité.

2<sup>o</sup> Essais d'électrodialyse et Répartition du phosphore. A titre d'exemple de la manière dont ces essais ont été exécutés, nous allons décrire plus en détail celui désigné par le N° I et qui a été effectué en dernier lieu. On fit usage pour cet essai de 650 cm<sup>3</sup> de la solution d'ovalbumine mentionnée précédemment (E p. 32), et qui, fraîchement préparée, avait été recristallisée six fois, puis dialysée; la teneur totale en N d'albumine était de 14<sup>g</sup>.645, phosphore total 115<sup>mg</sup>.05, donc en tout 7<sup>mg</sup>.86 de phosphore par g de N d'albumine.

Après deux jours d'abandon sans courant, on put déceler dans les deux liqueurs extérieures une trace infime de sulfate d'ammonium et point d'ovalbumine.

Après un jour d'électrodialyse (l'anode à gauche, 70—40 milliampères), il n'y avait pas d'albumine dans la liqueur de la cathode, tandis que celle de l'anode contenait env. 27 % de la totalité de l'albumine. (Marque: Anode v I).

Après avoir versé de l'eau pure dans les compartiments cathodique et anodique, on continua l'électrodialyse pendant un jour avec la même direction de courant (40—28 milliampères). La liqueur cathodique se montra exempte d'albumine, tandis que l'anodique renfermait 22 % env. de toute l'albumine primitive (Marque: Anode v. II).

Après rinçage soigneux des deux compartiments extérieurs avec de l'eau, on y versa de l'eau pure comme liqueurs cathodique et anodique respectivement, puis on abandonna l'appareil sans courant pendant deux jours. Au bout de ce temps, il n'y avait pas d'albumine dans l'ancien compartiment cathodique, tandis que l'autre compartiment en renfermait un peu (moins de 1 % de la quantité primitive d'albumine).

Ayant rincé et rempli à nouveau les deux compartiments, on continua l'électrodialyse pendant deux jours avec direction inverse du courant (22 milliampères). Ce temps écoulé, la

liqueur cathodique se montra privée d'albumine, et l'anodique en renfermait env. 8 % de toute la quantité primitive (Marque: Anode h. I.).

L'électrodialyse fut alors interrompue et la liqueur intérieure dépourvue par filtration d'une faible quantité d'albumine dénaturée (v. p. 39). A l'analyse, cette liqueur se trouva contenir env. 33 % de toute l'albumine primitive.

On a récupéré, au total, 90 % env. de l'albumine primitive. Le reste s'était perdu par les rinçages, les prélèvements d'échantillons etc.

Après avoir prélevé des échantillons pour l'analyse, on précipita par le sulfate d'ammonium, puis fit recristalliser trois fois, tant la liqueur intérieure (J) que les diverses liqueurs anodiques réunies (marquées A). Les solutions des produits recristallisés furent analysées et utilisées pour des déterminations de solubilité (v. p. 41).

Le relevé ci-dessous renseigne sur la répartition du phosphore; les nombres indiquent en mg le phosphore par g de N d'albumine.

Matière première, E.....	7.86
Liqueurs anodiques: v I.....	8.94
— — v II.....	8.26
— — h I.....	8.77
— — après recristallisation, A.....	8.53
Liqueur intérieure.....	6.71
— — après recristallisation, J.....	6.67

Tant pour la liqueur intérieure que pour l'une des liqueurs anodiques, nous avons dosé aussi bien le phosphore total que le «phosphore coagulable» et n'avons pu constater de différence entre eux; il paraîtrait donc qu'il n'y a pas eu séparation d'acide phosphorique, ni d'aucune autre combinaison phosphorée non coagulable.

Essai N° II. Cet essai a été fait sur un échantillon d'albumine d'œuf, M, fraîchement préparé, recristallisé six fois, puis dialysé. A la suite du traitement décrit plus haut, la plus grande partie de l'albumine passa au bout de deux jours dans la liqueur anodique I, puis presque tout le reste passa dans le courant de deux autres jours dans la liqueur anodique II; la partie restante de la liqueur intérieure ne renfermait qu'une dose infime d'albumine, dont on ne fit pas usage.

On précipita les liqueurs anodiques par le sulfate d'ammo-

nium et fit recristalliser les précipités trois fois; les solutions finalement obtenues ( $A_I$  et  $A_{II}$ ) furent soumises à l'analyse, puis employées à des déterminations de solubilités (v. p. 41).

La teneur en phosphore, exprimée en mg par g de N d'albumine, s'est chiffrée comme suit:

Matière première, M.....	7.63
Liqueur anodique, I, recristallisée $A_I$ .....	8.50
— — — II, — $A_{II}$ .....	6.20

Le taux peu élevé de phosphore en  $A_{II}$  est sans doute imputable à ce que les complexes riches en phosphore auraient passé dans la première liqueur anodique, de sorte que la composition de la seconde liqueur anodique correspond à peu près à la composition normale de la liqueur intérieure.

Essai No III. Cet essai a porté sur une solution relativement faible d'un échantillon d'albumine, D.  $\mathcal{A}E$ . 24, fraîchement préparé, recristallisé six fois, puis dialysé. Les membranes employées étaient fortement séchées et de ce fait peu pénétrables; par conséquent, la solution d'albumine étant très pauvre en électrolyte et l'intensité du courant se chiffrant seulement par 30—10 milliamperes, il n'a passé qu'assez peu d'albumine dans la liqueur anodique, dont on changeait journallement pendant les neuf jours que dura l'électrodialyse.

On n'est pas parvenu à tirer une albumine cristalline des liqueurs anodiques fortement diluées.

Dans la liqueur intérieure, grâce à l'électrodialyse prolongée, il s'était déposé un précipité passablement abondant qui, séparé par filtration, se trouva constitué par de l'albumine dénaturée (v. p. 40). On précipita la liqueur intérieure par le sulfate d'ammonium et fit recristalliser trois fois le précipité; ensuite, la solution finalement obtenue (J) fut analysée et servit à des déterminations de solubilité (v. p. 41).

La teneur en phosphore exprimé en mg par g de N d'albumine, s'est chiffrée ainsi:

Matière première, D. $\mathcal{A}E$ . 24.....	7.50
Liqueur intérieure, recristallisée, J.....	4.82

Le taux minime de phosphore dans J montre que la dialyse très prolongée a fait passer dans les liqueurs anodiques une partie considérable des complexes phosphorés.

Essai N° IV. Pour cet essai on a fait emploi d'un échantillon d'albumine dialysée, D.Æ. 15, qui avait été abandonné depuis huit ans en glacière et avait déposé une partie de l'albumine à l'état dénaturé. Après séparation de celle-ci par filtration, on soumit le filtrat à l'électrodialyse comme décrit ci-dessus; durée totale de l'électrodialyse 36 heures; trois changements de la liqueur anodique, avec une intensité de courant de 40—20 milli-ampères, de sorte que seulement une faible partie de l'albumine fut transportée dans les trois liqueurs anodiques. Les liqueurs anodiques furent réunies, et l'on précipita par le sulfate d'ammonium le mélange de même que la liqueur intérieure. Après avoir fait recristalliser trois fois les précipités, on fit l'analyse des solutions finalement obtenues (A et J) et les employa pour des déterminations de solubilité (v. p. 41).

Teneur en phosphore, exprimée en mg par g de N albumine:

Liqueurs anodiques, recristallisées, A ..... 5.70

Liquueur intérieure, recristallisée, J ..... 4.69

La teneur en phosphore de la matière première n'était pas connue; cependant, vu qu'il s'agit d'un échantillon dialysé conservé dans de la glace durant de nombreuses années, il est hors de doute (v. Section B e p. 45) que cette teneur a été peu considérable. La teneur en phosphore si peu élevée de la liqueur anodique paraît ainsi s'expliquer d'une façon naturelle et plausible.

Il résulte de ces essais qu'il est possible par électrodialyse de fractionner l'ovalbumine en une partie relativement riche en phosphore et qui passe à travers la membrane de collodion anodique, et une partie relativement pauvre en phosphore, laquelle reste dans la liqueur intérieure. Non seulement les solutions d'ovalbumine fraîchement préparées, mais encore de vieux échantillons pauvres en phosphore, peuvent être fractionnés de cette manière.

3° Dépôt dans la liqueur intérieure. Dans ses recherches sur la préparation de substances protéiques dépourvues d'électrolytes et solubles dans l'eau, Wo. Pauli<sup>1)</sup> insiste sur ce fait que, au moyen d'une électrodialyse convenable, il est possible d'en séparer la totalité de la globuline sous forme d'une

<sup>1)</sup> V. p. ex.: M. Adolf et Wo. Pauli: Biochem. Zeitschr., **152**, 360 (1924).

fraction insoluble dans l'eau. On peut ensuite se demander si le faible dépôt que nous toujours avons vu apparaître à la suite de l'électrodialyse de solutions d'ovalbumine, n'était pas formé de globuline d'œuf. S'il en était ainsi, il s'ensuivrait que, en tout cas sur ce point, le procédé habituellement suivi pour la préparation des solutions d'ovalbumine pures n'est pas adéquat. Dans les cas où nous avons eu à notre disposition des quantités de dépôt tant soit peu abondantes, il a donc fallu entreprendre l'examen de cette question.

C'est dans l'essai N° III que le dépôt est apparu dans la plus grande proportion (v. p. 38). Séparé par filtration, il fut, sur le filtre même, lavé une seule fois avec de l'eau, puis seringué dans un verre de centrifuge, où on le broya bien avec 25 cm<sup>3</sup> d'eau, pour le séparer ensuite par centrifugation. Le traitement à l'eau fut répété en tout quatre fois. Dans les deux dernières eau de lavage, on ne put déceler d'azote par dosage d'après Kjeldahl. Replacé dans le verre de centrifuge, le dépôt fut alors broyé et délayé avec 25 cm<sup>3</sup> d'une solution de chlorure de sodium norm. au  $\frac{1}{10}$ , puis centrifugé de nouveau. Dans la solution de sel marin, on ne trouva, en tout, que 0mg.09 de N total et, par conséquent, le dépôt ne renfermait en tout cas que des traces de globuline non dénaturée.

Le dépôt restant fut ensuite délayé avec 25 cm<sup>3</sup> d'acide sulfurique 2-norm.: dans 10 cm<sup>3</sup> de cette suspension on constata une teneur de 17mg.28 en N total, dans une autre portion de 10 cm<sup>3</sup>, 0mg.129 de phosphore total, donc 7mg.47 de phosphore par g de N total. Ainsi, on n'est pas en présence, ici non plus, de globuline dénaturée, dont le taux de phosphore est beaucoup plus bas (v. p. 27), mais bien d'ovalbumine dénaturée ayant une teneur parfaitement normale en phosphore.

Le dépôt beaucoup plus petit provenant de l'essai N° I fut examiné de la même manière. Ni dans les extraits aqueux ni dans celui fait à l'aide d'une solution de sel marin, on ne put reconnaître la présence d'azote; le dépôt resté indissous accusa un taux de phosphore s'élevant à 9mg.44 par g de N total; cependant, à cause de l'insuffisance quantitative des matières analysées, les résultats sont certainement entachés de graves erreurs.

4<sup>o</sup> Essais de solubilité. Ainsi qu'il a été indiqué à



l'occasion de chaque essai d'électrodialyse, les estimations de solubilité ont été effectuées sur les solutions suivantes:

Essai N° 1 .....	A og J
— - II.....	A <sub>I</sub> og A <sub>II</sub>
— - III.....	J
— - IV.....	A og J

Les résultats de ces essais de solubilité, qui furent exécutés de la manière décrite précédemment, se trouvent groupés dans le Tableau III, qui se passe de commentaires.

Dans les trois dernières colonnes verticales de ce tableau, les valeurs établies expérimentalement sont rapportées à  $p_{AII} = 4.90$ , et les valeurs ainsi calculées et corrigées de  $\log s$  et de  $s$  sont insérées, par comparaison avec la solubilité normale, sur les courbes étalons (Fig. 6 et 7).

Un coup d'oeil sur ces deux figures permet de voir que tous les échantillons examinés ont une solubilité plus grande que la normale; toutefois, l'écart avec celle-ci se montre bien minime dans tous les cas où il s'agit d'échantillons dont la teneur en phosphore ne s'écarte pas trop du taux ordinaire (IA, IJ, II A<sub>I</sub> et, en partie, II A<sub>II</sub>). Lorsque cependant la quantité de phosphore tombe en dessous de 6 mg par g d'albumine, on voit la solubilité s'accroître, et d'autant plus que la teneur en phosphore est plus faible ((IV A. III J et IV J)); pour ce qui regarde ce dernier fait, nous renvoyons aux considérations émises dans l'introduction (v. p. 8 et 16).

Enfin, il est à remarquer que, comme le montre la Fig. 6, la ligne droite qui peut être tracée par les cinq points représentant les essais faits sur l'échantillon le plus facilement soluble (IV J), a un degré d'inclinaison légèrement différent du normal.

#### e. Echantillons vieillis.

L'objet de ce chapitre est de rendre compte de la teneur en phosphore et de la solubilité de quelques échantillons d'ovalbumine qu'on avait conservés, saturés de toluène, pendant plusieurs années dans une glacière, où ils étaient couverts de glace. Nous ferons mention de cinq échantillons, dont quatre avaient été abandonnés en solution à l'état dialysé, le cinquième au contraire sous forme de dépôt cristallisé, recouvert de l'eau-mère riche en sulfate d'ammonium.

Tableau III.

Solution d'ovalbumine employée	Solution d'ovalbumine électrodialysée		Numéro d'essai	Pour 100 g d'eau		Activité d'ions hydro- gène du filtrat $p_{aH}$	$\log s$	$\log s$ rapporté à $p_{aH} = 4.90$		$s$ $\left(\begin{smallmatrix} \text{corr.} \\ 4.90 \end{smallmatrix}\right)$
	Marque	Teneur en phosph. mg l (par 1 g N prot.)		Hydrate d'œuf s	Sulfate d'ammonium s			Corr. $-1.7(p_{aH} - 4.90)$	$\log s$ $\left(\begin{smallmatrix} \text{corr.} \\ 4.90 \end{smallmatrix}\right)$	
E (fraiche- ment préparé)	I A ○	8.53	1	0.961	24.754	4.88	-0.017	0.034	0.017	1.040
			2	0.653	25.770	4.92	-0.185	-0.034	-0.219	0.604
			3	0.369	26.862	4.91	-0.433	-0.017	-0.450	0.355
			4	0.209	27.903	4.87	-0.680	0.051	-0.629	0.235
			5	0.125	29.031	4.91	-0.903	-0.017	-0.920	0.120
	I J △	6.67	6	1.214	24.812	4.95	0.084	-0.085	-0.001	0.998
			7	0.799	25.789	4.99	-0.097	-0.153	-0.250	0.562
			8	0.497	26.904	4.99	-0.304	-0.153	-0.457	0.349
			9	0.311	27.949	5.01	-0.507	-0.187	-0.694	0.202
M fraiche- ment préparé)	II A <sub>I</sub> ⊙	8.50	10	0.448	25.916	4.85	-0.349	0.085	-0.264	0.545
			11	0.279	26.902	4.85	-0.554	0.085	-0.469	0.340
			12	0.172	28.058	4.87	-0.764	0.051	-0.713	0.194
	II A <sub>II</sub> ⊙	6.20	13	0.343	26.848	4.84	-0.465	0.102	-0.363	0.434
			14	0.543	25.764	4.83	-0.265	0.119	-0.146	0.715
D. Æ. 24 (fraiche- ment préparé)	III J □	4.82	15	1.018	26.973	5.01	0.008	-0.187	-0.179	0.662
D. Æ. 15 (âge de 8 ans)	IV A △	5.70	16	0.445	26.903	4.84	-0.352	0.102	-0.250	0.562
			17	1.388	24.861	4.80	0.142	0.170	0.312	2.051
	IV J △	4.69	18	0.910	25.848	do.	-0.041	0.170	0.129	1.346
			19	0.578	26.910	do.	-0.238	0.170	-0.068	0.855
			20	0.378	28.022	do.	-0.423	0.170	-0.253	0.558
			21	0.268	28.951	do.	-0.572	0.170	-0.402	0.396

1° Teneur en phosphore.

Échantillon I.D.Æ.4. La solution d'ovalbumine fut préparée, dialysée et analysée à la manière ordinaire, au mois de mars 1915; on en utilisa une partie, abandonnant le reste saturé

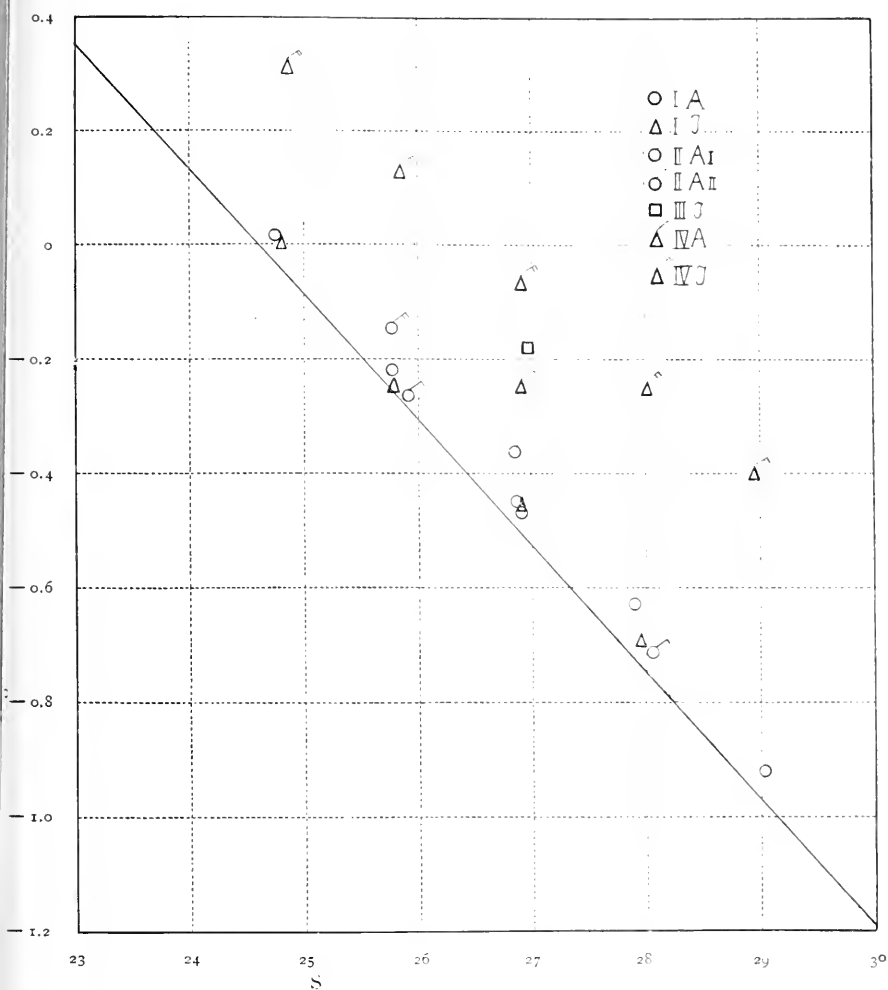


Fig. 6.

de toluène en glace dans la glacière. Au bout de deux ans à peu près (jusqu'en janvier 1917), il se trouva en grande partie dénaturé; après séparation par filtration de cette partie, puis analyse du filtrat, on en employa une portion pour la mesure de la pression osmotique de l'ovalbumine contenue dans la solution; cette pression fut reconnue pour normale. Le reste de la solution fut abandonné de nouveau en glace dans la glacière. En novembre 1925, on sépara par filtration le dépôt qui s'était formé dans l'intervalle et qu'on lava soigneusement avec de l'eau. Enfin,

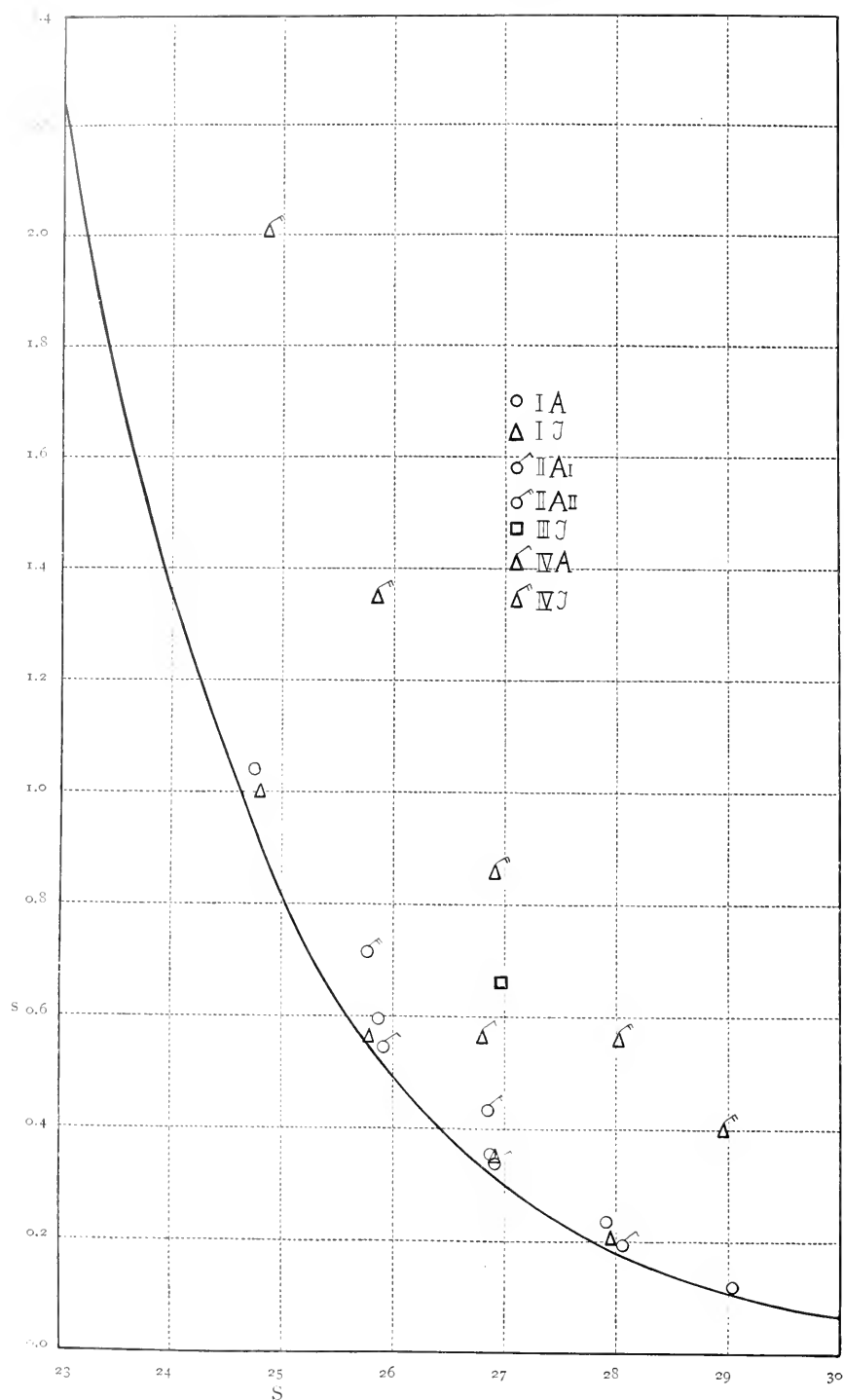


Fig. 7.

on a soumis à l'analyse et la liqueur filtrée et le dépôt<sup>1)</sup>, avec les résultats indiqués ci-après.

100 cm<sup>3</sup> de solution contenaient:

		g de N d'alb.	g de N de NH <sub>3</sub>	mg de P par g de N d'alb.	P <sub>all</sub>
En mars	1915	4.160	0.0049	—	4.88
- janvier	1917	1.766	—	—	5.43
- nov.	1925	1.545	0.0250	9.2	5.78

L'ensemble du dépôt séparé par filtration en novembre 1925, se trouva renfermer 0g.338 de N d'albumine, et pour chaque g de N d'alb. on trouva 6mg.6 de P.

Il ressort de ces données analytiques que plus de 60% de l'ovalbumine a subi la dénaturation par abandon durant dix ans de cette solution dialysée et partant très pauvre en électrolytes; en même temps, sa teneur en ammoniacque s'est accrue sinon fortement, du moins d'une façon appréciable. Par contre, il ne paraît pas y avoir eu formation d'autres produits de dégradation: après dénaturation d'une partie de la solution au moyen de l'alcool, on a dosé, dans le filtrat du dépôt ainsi formé, tant le N total que N ammoniacal, et pour 100 cm<sup>3</sup> de la solution obtenue en novembre 1925 ces estimations ont donné:

0g.0264 de N total  
0g.0251 de N de NH<sub>3</sub>.

On voit, en outre, qu'au cours de ce long repos la réaction de la solution est devenue de moins en moins acide. Il n'y a pas moyen d'établir avec certitude si cette modification est due à l'accroissement de la quantité d'ammoniacque, ou bien si d'autres facteurs — tels que par ex. une incorporation d'alcali provenant de la bouteille de conservation — sont entrés en jeu.

Pour ce qui concerne la teneur primitive en phosphore de la solution d'ovalbumine, nous n'en savons rien non plus; cependant, en supposant que le dépôt formé avant janvier 1917 et alors séparé par filtration ait contenu 6mg.6 de P par g de N d'albumine de même que celui séparé par filtration en novembre 1925,

<sup>1)</sup> Ces dernières analyses ainsi que celles rapportées dans la suite et marquées (Perlzweig), ont été exécutées par M. le Dr. W. A. Perlzweig, de Johns-Hopkins Hospital, à Baltimore (États Unis d'Amérique), lors d'un séjour d'études à notre laboratoire.

on peut estimer comme suit, approximativement, le taux primitif de phosphore de l'albumine:

De 4<sup>g</sup>.160 de N d'albumine, il n'en reste à l'état non dénaturé que 1<sup>g</sup>.545: donc, la quantité dénaturée totale s'élève à 2<sup>g</sup>.615: par conséquent, on aura:

$$x \cdot 4.160 = 9.2 \cdot 1.545 + 6.6 \cdot 2.615.$$

Il en résulte  $x = 7.6$ , c. à. d. un taux de phosphore parfaitement normal.

On voit par là que, par suite de la dénaturation, l'ovalbumine a abandonné une faible quantité d'une matière phosphorée, qui se retrouve dans la solution, dont la richesse en phosphore est conséquemment tout particulièrement élevée, savoir 9<sup>mg</sup>.2 P par g N d'albumine. — Cependant, ce n'est pas seulement l'albumine dénaturée qui a perdu partiellement son phosphore: il en est de même, et à un degré encore plus haut, de l'albumine existant dans la solution. En effet, si l'on précipitait cette albumine au moyen du sulfate d'ammonium, puis qu'on fit recristalliser le précipité trois fois de la manière ordinaire, ce qui n'a présenté aucune sorte de difficulté, l'albumine ainsi obtenue accusa une teneur en phosphore de 5<sup>mg</sup>.4 seulement par g de N d'albumine.

Échantillon II. D. Æ. 3. Cette solution d'ovalbumine était encore plus vieille que celle mentionnée ci-dessus: elle avait été préparée, dialysée et analysée en décembre 1914; depuis cette époque, un reste de cet échantillon était conservé avec du toluène en glace dans une glacière; il fut filtré en novembre 1925, et le filtrat ainsi que le dépôt lavé furent analysés (Perlzweig).

100 cm<sup>3</sup> de solution contenaient:

	g de N d'alb.	g N de NH <sub>3</sub>	mg P par g N d'alb.	p <sub>aH</sub>
Décembre 1914	3.790	0.0013	—	4.81
Novembre 1925	2.064	0.0070	9.3	4.96

Dans le dépôt séparé par filtration, on trouva, en tout, 1<sup>g</sup>.450 N d'albumine, et pour chaque g N d'alb. 4<sup>mg</sup>.4 P.

Comme l'on voit, il s'est produit ici encore une séparation d'ammoniaque, et en même temps la réaction est devenue moins acide; toutefois, les changements survenus sont moins considérables que dans le cas précédent, de même que la partie dénaturée de l'ovalbumine s'élève à peine à la moitié. Par contre, la séparation de phosphore a été plus grande, car le dépôt n'en contenait que

Tableau IV.

Échantillon d'ovalbumine				Quantité dénaturée pendant le repos, pour- centages de la quantité pri- mitive.	Teneur en phos- phore exprimée en mg P par g de N d'albumine	
N°	Marque	Date de pré- paration	Age au moment du traitement		dans le dépôt dé- nature	dans l'ov- albumine recristal- lisée tirée de la solu- tion
I	D. Æ. 4	Mars 1915	env. 10 ans $1/2$	env. 60 %	6.6	5.4
II	D. Æ. 3	Déc. 1914	- 11 —	— 50 —	4.4	4.7
III	D. Æ. 16 B	Oct. 1917	- 8 — $1/2$	— 15 —	5.7	4.7
IV	D. Æ. 22	Nov. 1920	- 5 — $1/2$	— 5 —	6.1	6.7
V	I. D. Æ. 8	Déc. 1915	- 10 —	— 1 —	7.1	7.3

4<sup>mg</sup>,4 par g N d'albumine et une proportion semblable: 4<sup>mg</sup>,7 P par g N d'alb. fut constatée dans l'ovalbumine existant dans la liqueur filtrée, après précipitation de celle-ci par le sulfate d'ammonium, recristallisation trois fois répétée de la façon ordinaire, ce qui ne présenta pas de difficultés ni dans le cas présent ni dans ceux que nous allons mentionner.






De même que précédemment, on peut juger que l'ovalbumine primitive aura eu une teneur normale en phosphore, vu que l'équation

$$x \cdot 3.790 = 9.3 \cdot 2.064 + 4.4 (3.790 \div 2.064)$$

donne  $x = 7.1$ .

Échantillon III. D. Æ. 16 B. On avait préparé et dialysé cet échantillon d'ovalbumine en octobre 1917; lors de la filtration au mois de mars 1926, il était donc âgé de 8 ans  $1/2$  à peu près. La partie dénaturée n'était que de 15 % de la quantité totale; au contraire, la séparation de phosphore se trouva relativement considérable, car le dépôt séparé par filtration puis lavé, n'en renfermait que 5<sup>mg</sup>,7 par g N d'albumine. La liqueur filtrée contenait 7<sup>mg</sup>,9 P total par g N total, mais seulement 5<sup>mg</sup>,0 P coagulable par g N d'albumine coag., et l'on put constater exactement le même taux de phosphore et d'azote lorsque les échantillons employés aux dosages étaient coagulés à l'aide de l'alcool au lieu de la coagulation habituelle par chauffage. En bon accord avec ce résultat, l'ovalbumine précipitée du filtrat et recris-

Tableau V.

Désignation et âge	Solution d'ovalbumine employée	Marque	Teneur en phosph. mg P (par 1 g N prot.)	Numéro d'essai	Pour 100 g d'eau :		Activité d'ions hydrogène du filtrat $\text{pa}_\text{H}$	$\log s$	log s rapporté à $\text{pa}_\text{H} = 4.90$		$s$ (corr. 4.90)
					Hydrate d'œuf $s_1$	Sulfate d'ammonium $s_2$			Corr. - 1.7 ( $\text{pa}_\text{H} - 4.90$ )	$\log s$ (corr. 4.90)	
D. Æ. 4 env. 10 ans $1/2$	I		5.4	1	0.592	25.702	4.86	-0.228	0.068	-0.160	0.692
				2	0.933	24.651	4.85	-0.030	0.085	0.055	1.135
D. Æ. 3 env. 11 ans	II		4.7	3	0.478	26.537	4.80	-0.321	0.170	-0.151	0.706
D. Æ. 16 B env. 8 ans $1/2$	III		4.7	4	0.827	25.863	4.86	-0.082	0.068	-0.014	0.968
				5	0.522	26.788	4.87	-0.282	0.051	-0.231	0.587
				6	0.321	27.988	4.88	-0.493	0.034	-0.459	0.348
				7	1.098	24.780	4.92	0.041	-0.034	0.007	1.016
D. Æ. 22 env. 5 ans $1/2$	IV		6.7	8	0.717	25.674	4.93	-0.144	-0.051	-0.195	0.638
				9	0.450	26.710	4.94	-0.347	-0.068	-0.415	0.385
				10	0.279	27.814	4.95	-0.554	-0.085	-0.639	0.230
				11	0.171	28.869	4.95 (incert.)	-0.767	-0.085	-0.852	0.141
I. D. Æ. 8 env. 10 ans	V		7.3	12	1.425	24.868	4.85	0.154	0.085	0.239	1.734
				13	0.881	25.851	4.85	-0.055	0.085	0.030	1.072
				14	0.545	26.787	4.88	-0.264	0.034	-0.230	0.589
				15	0.346	27.983	4.87	-0.461	0.051	-0.410	0.389
				16	0.220	28.953	4.88	-0.658	0.034	-0.624	0.238

tallisée quatre fois accusa une teneur en phosphore de 4<sup>mg</sup>.7 par g N d'albumine.

Échantillon IV. D. Æ. 22. Un échantillon encore plus jeune d'ovalbumine dialysée, préparé en novembre 1920 et qu'on avait laissé au repos durant 5 ans  $1/2$  environ, n'avait déposé pendant ce temps, sous la forme dénaturée, que 5 % env. de la quantité totale d'albumine. Le dépôt renfermait 6<sup>mg</sup>.1 P par g N d'albumine, et la liqueur filtrée contenait 7<sup>mg</sup>.2 P total par g N total, mais seulement 6<sup>mg</sup>.8 P coagulable par g N coag., que la coagulation eût été faite par chauffage ou bien à l'aide de l'alcool. En parfait accord avec ce résultat, l'ovalbumine précipitée du filtrat et recristallisée trois fois, accusa une teneur en phosphore de 6<sup>mg</sup>.7 par g N d'albumine.



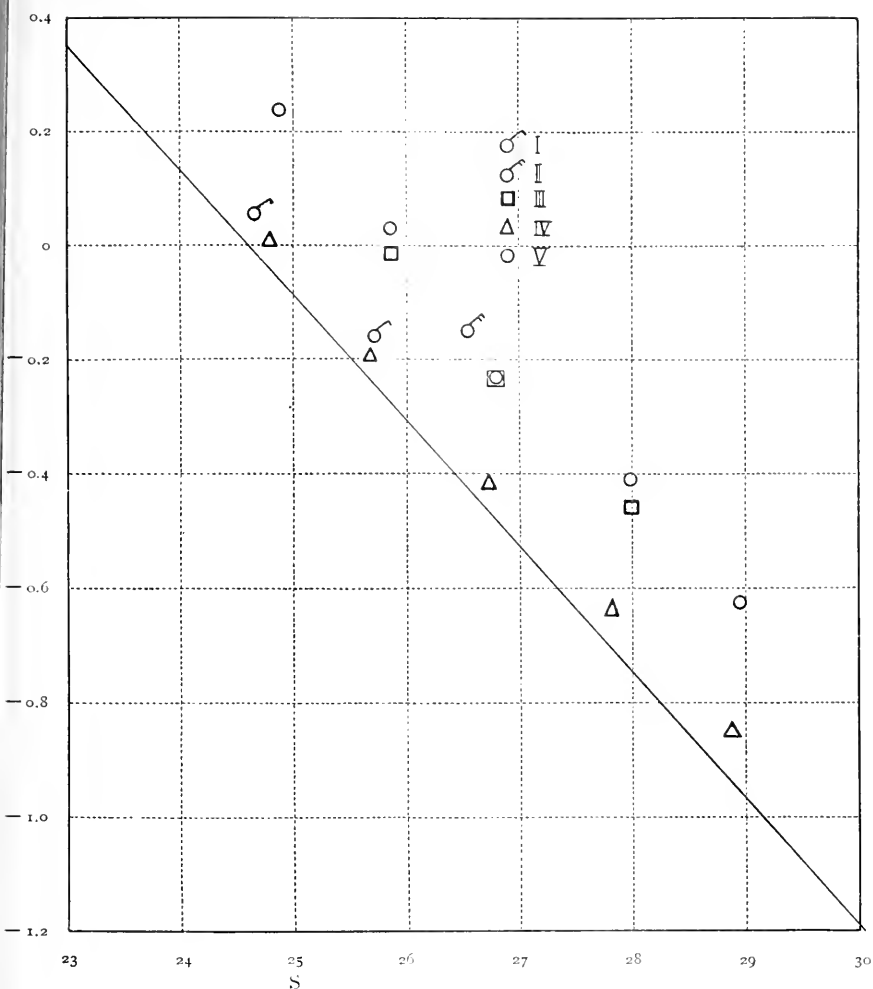


Fig. 8.

Échantillon V. I. D. Æ. 8. Enfin, il convient de mentionner un échantillon préparé en décembre 1915, non dialysé, qu'on avait conservé saturé de toluène en glace dans la glacière durant une dizaine d'années. Dans 100 cm<sup>3</sup> de la solution obtenue à partir du dépôt, on trouva environ 24 g d'hydrate d'ovalbumine et 12 g de sulfate d'ammonium. Cet échantillon non dialysé n'avait guère éprouvé de changement pendant le long abandon. 1 % env. de la totalité de l'albumine s'était dénaturé, et le dépôt dénaturé contenait 7<sup>mg</sup>.1 P par g N d'albumine. La liqueur filtrée accusa 7<sup>mg</sup>.5 P par g d'albumine, que la coagulation fût opérée

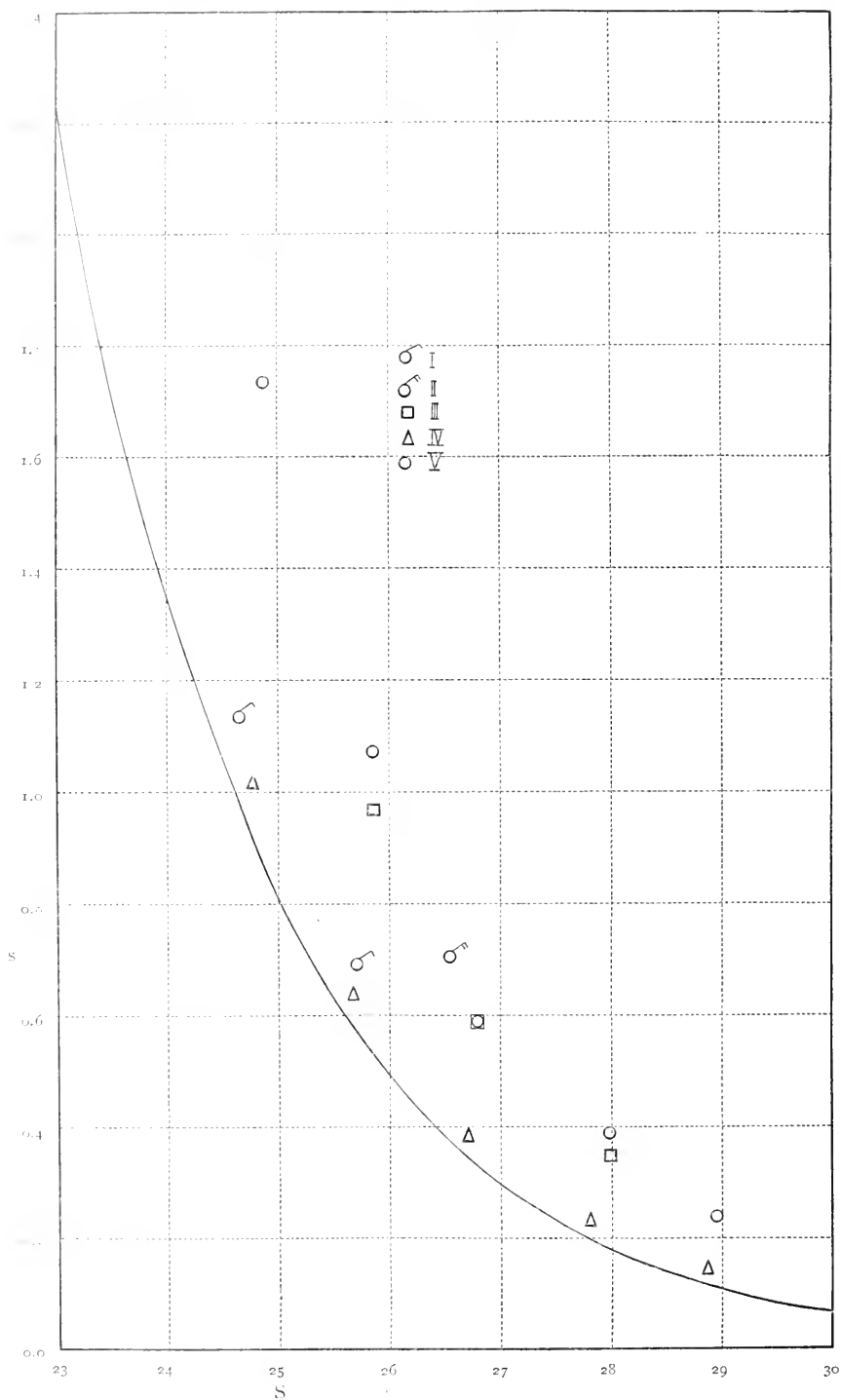


Fig. 9.

par chauffage ou au moyen de l'alcool. En bon accord avec ce résultat, l'ovalbumine précipitée du filtrat et recristallisée quatre fois montra une teneur en phosphore de  $7^{mg}.3$  par g N d'albumine.

Le Tableau IV donne un aperçu de la répartition telle qu'elle a été constatée par l'examen des cinq albumines vieilles susmentionnées.

2° Essais de solubilité. Sur chacune de nos cinq ovalbumines recristallisées, des essais de solubilité ont été effectués par le procédé ci-dessus décrit, avec les résultats rassemblés au Tableau V.

Dans les trois dernières colonnes verticales de ce tableau, les valeurs expérimentalement trouvées sont rapportées à  $pa_{II} = 4.90$ , et les valeurs ainsi calculées et corrigées de  $\log s$  et de  $s$  sont insérées sur les courbes étalons (Fig. 8 et 9), par comparaison avec les solubilités normales.

Il appert de ces figures que tous les échantillons examinés possèdent une solubilité sensiblement plus grande que la normale. Pour ce qui concerne les quatre premiers échantillons, qui proviennent tous de vieilles solutions d'ovalbumine dialysées, il semble exister une relation entre la teneur en phosphore et la solubilité, puisque les échantillons II et III très pauvres en phosphore présentent une solubilité notablement plus grande que les échantillons I et IV, dont la solubilité se rapproche de la normale. Il en est tout autrement de l'échantillon V, lequel provient d'un échantillon d'ovalbumine non dialysée qui avait été abandonné à l'état cristallisé durant une dizaine d'années. Il n'y a guère eu ici ni dénaturation ni séparation de complexes phosphorés (v. Tableau IV), mais il doit tout de même s'être passé quelque chose, car malgré la teneur normale en phosphore la solubilité de cet échantillon s'est trouvée notablement plus grande que la normale.

## ÉPILOGUE.

Après que le manuscrit du présent mémoire avait été achevé, il a paru un travail de Leslie Frank Hewitt intitulé: «Optical rotatory power and dispersion of proteins»<sup>1)</sup>, qui traite, entre

<sup>1)</sup> Biochem. Journ., **21**, 216 (1927).

autres, de la question de l'influence exercée par les lipoides sur le pouvoir rotatoire des albumines et des globulines.

Pour la purification de ces substances, Hewitt a appliqué, sous une forme un peu modifiée, le procédé de Hardy et Gardiner<sup>1)</sup> mentionné précédemment, et par là il a réussi à traiter un échantillon d'ovalbumine par l'alcool et l'éther à froid, sans que l'albumine en ait été dénaturée. L'albumine s'est dissoute dans l'eau en formant une solution limpide, où l'on a pu provoquer la cristallisation de la manière ordinaire, «but with some difficulty». Ce qui nous intéresse tout particulièrement ici, c'est que Hewitt n'a pas pu non plus extraire les constituants phosphorés de l'ovalbumine, qui présentait un taux de phosphore tout à fait normal (savoir 7<sup>mg</sup>.7 par g N d'albumine).

Hewitt trouve à l'albumine ainsi traitée un pouvoir rotatoire de  $[\alpha]_{5461}^{20^0} = \div 44^0.5$ , valeur qui est de 20 % env. plus élevée que celles constatées par Young<sup>2)</sup> et H. Jessen-Hansen<sup>3)</sup> pour des ovalbumines non traitées mais d'ailleurs bien purifiées. Cette différence semble trop grande pour pouvoir être imputable à la présence de lipoides susceptibles d'être éliminés par le traitement à l'alcool et l'éther. La concentration en ions hydrogène de la solution — facteur dont Hewitt ne dit rien dans son mémoire et qui pourtant, selon les recherches de Jessen-Hansen, exerce une influence appréciable sur le pouvoir rotatoire — est peut-être pour quelque chose dans la différence en question, mais ne suffit sans doute pas à l'expliquer entièrement. D'un autre côté, il semble y avoir possibilité que le pouvoir rotatoire plus élevé observé par Hewitt soit dû à un commencement de dénaturation. La faculté réduite de cristallisation fait soupçonner qu'il en est ainsi, et Hewitt lui-même a d'ailleurs démontré que le pouvoir rotatoire de la sérum-albumine s'accroît fortement dès qu'une dénaturation se déclenche (soit par l'action de l'alcool à la température ordinaire, soit par abandon en solution relativement acide ou par chauffage). Cette question est du reste d'une grande importance pour l'explication de la nature du processus de coagulation, au sujet duquel fort peu est connu actuellement.

<sup>1)</sup> Journ. Physiol., **40**, 68 (1910).

<sup>2)</sup> Proc. Roy. Soc. London B, **93**, 15 (1922).

<sup>3)</sup> Compt. rend. Laboratoire Carlsberg, **16**, N° 10 (1927).

Si cependant il venait à être démontré que le processus de dénaturation commence, ou bien soit accompagné, par une modification notable du pouvoir rotatoire, une pareille constatation viendrait à l'appui, ce nous semble, de cette hypothèse que dans le cours de la dénaturation il se produit des transpositions intramoléculaires.

*Avril 1927.*



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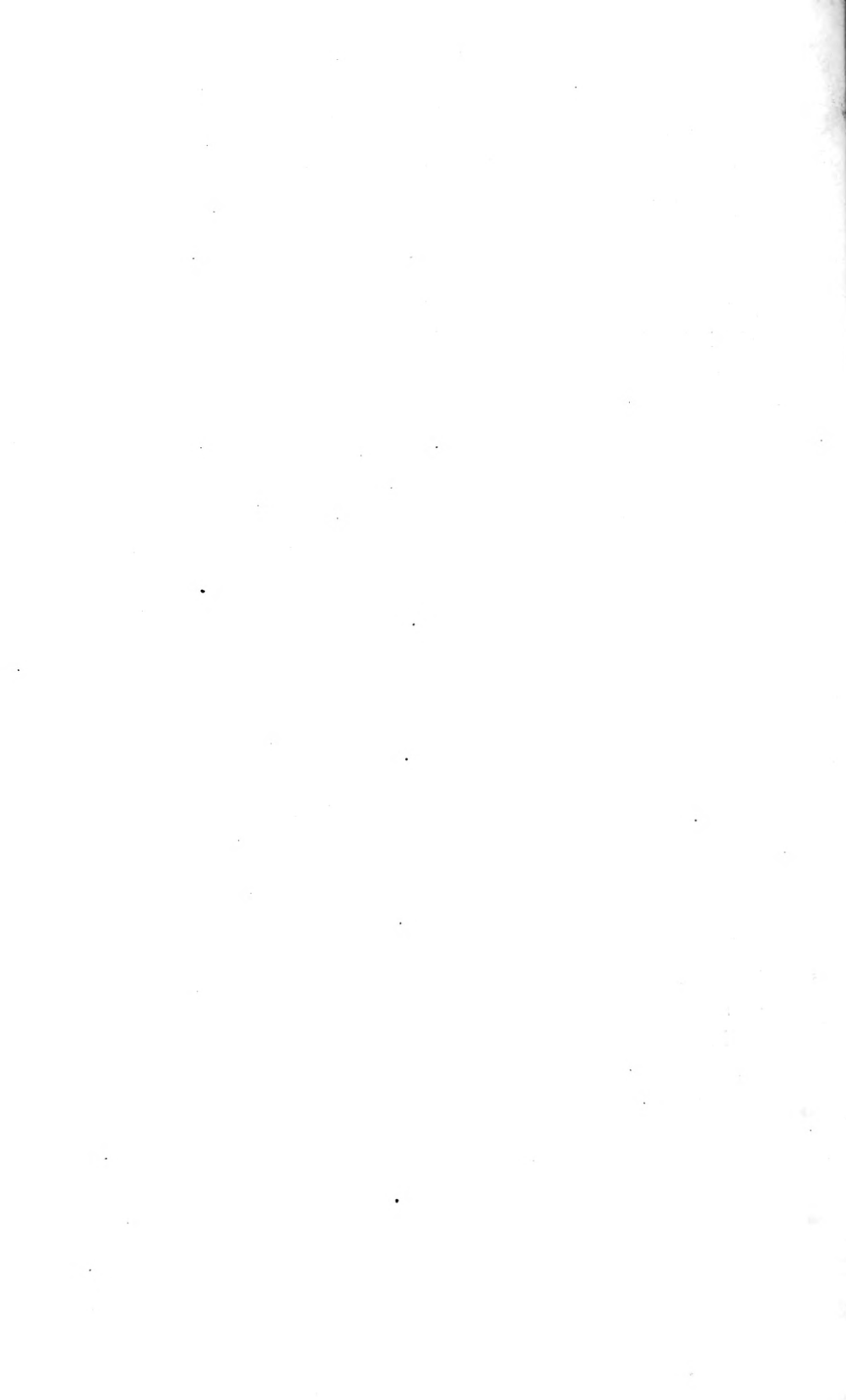
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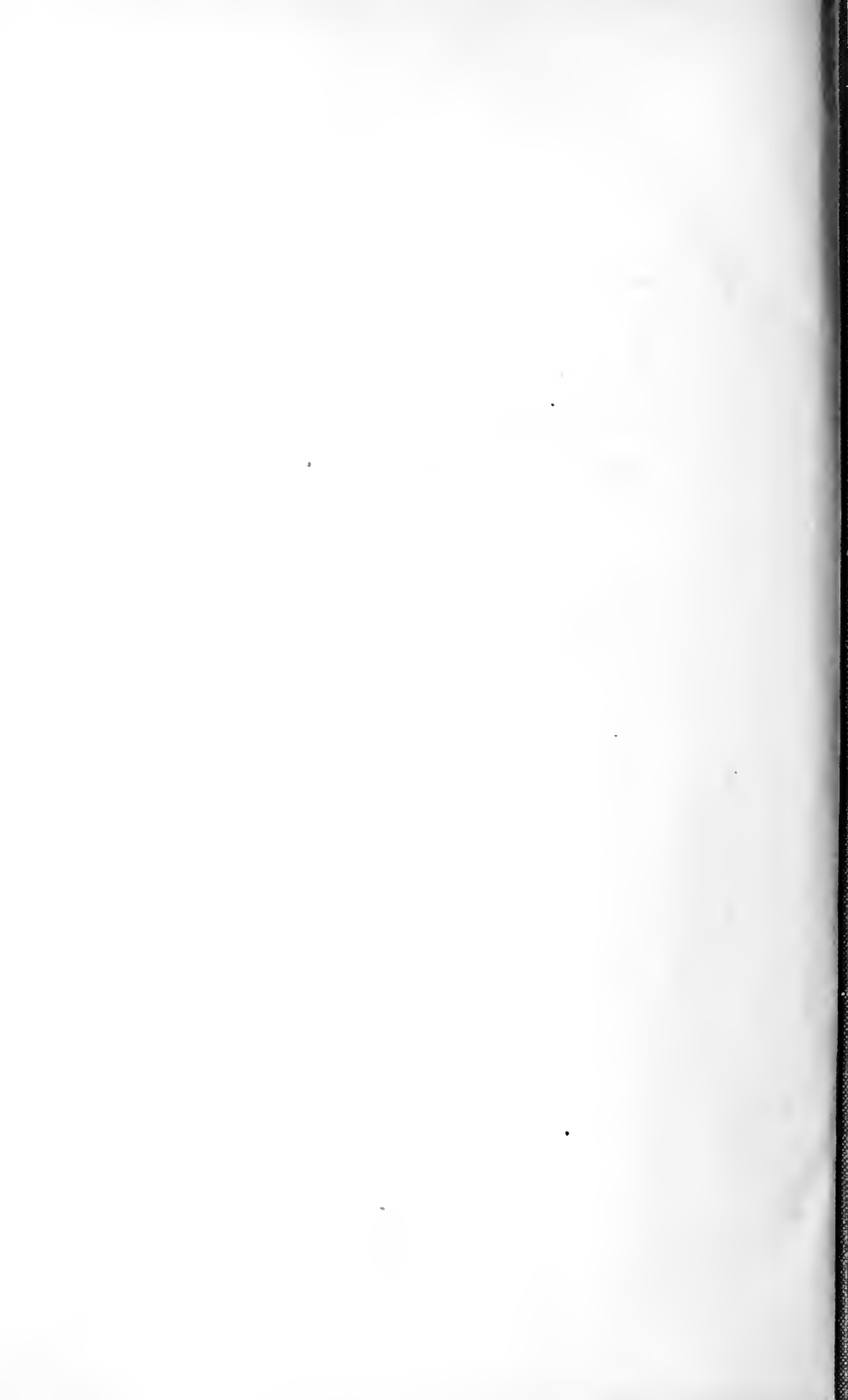












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